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The Journal of HEREDITY

This Number is Devoted to the Discussion of the Effects of X-Rays and Radium in Producing Changes in Genes and Chromosomes

Ray-Induced Giant and Dwarf Tobacco Plants

VOLUME 20 JUNE, 1929 NUMBER 6

The American Genetic Association

An incorporated organization devoted to promoting a knowledge of the laws of heredity and their application to the improvement of plants, animals, and human racial stocks

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The

Journal of Heredity

Vol. XX JUNE, 1929 No. 6

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"POP AND THE KIDS"—RAY-INDUCED VARIATIONS

Frontispiece

Three tobacco plants produced by using pollen from flowers treated with X-rays. The seed parent was not treated. The plant in the center is much larger and more vigorous than the control, the other two were very much dwarfed. All three are fertile. (*N. tabacum* var. *purpurea.*) (Photograph from Science Service.)

A NUMBER DEVOTED TO RADIATION-INDUCED MUTATIONS

THE discovery of X-rays in 1895 and of radium in 1898 gave the world of science unique new tools that have been used in such widely different ways as to alleviate suffering and to afford valuable clues as to the constitution of matter. In the field of medicine it was soon found that this new aid in diagnosis and therapeutics carried with its beneficent effects grave dangers to patients and operators, and some of the pioneers in the medical use of the rays even paid with their lives for unwittingly exposing themselves too freely to their influence. The resulting burns and the other effects of the rays on various tissues, organs and tumor growths indicated that they exerted a profound influence on the organism and on the cell.

Not until a generation later was proof obtained that these same rays produced permanent changes in the very mechanism of heredity—the genes themselves, and Muller's announcement in 1927 of the *Transmutation of the Gene* reported the first successful attempt to reach within the cell to alter these tiny particles that have gained a growing importance as the carriers of heredity. So startling a discovery has resulted in intense activity in this new field of research. This has amply verified Muller's results, and has brought to light new ways to use X-rays in facilitating genetic research, through altering and rearranging chromosomes as well as genes. Other organisms have been exposed to the rays, and significant differences in their reaction have been revealed. The papers here presented summarize some of the results obtained in less than two years. Such remarkable progress in so short a time is a tribute to the investigators and promises much for the future.

As originally projected, this series was to be complete in a single number of the Journal, or as two series covering animals and plants. Unfortunately some of the contributors have been delayed in the preparation of their manuscripts, and it has seemed best to proceed with the publication of the papers so far submitted, without attempting to classify them as "botanical" or "zoological". The others will be published as soon as possible, and it is regretted that all could not be presented at one time. It is perhaps worthy of note that this issue of the Journal, containing sixty-four pages, is the largest ever issued.

The editor has noted in some quarters a feeling that the inclusion of so many "technical" articles in one number was working a hardship on members of the A. G. A. who may not have been following closely this field of research. In spite of this he declines to be too apologetic, but he hastens to comfort those who take this view by assuring them that the present number represents a temporary and not a "germinal" change. The subject of heredity is becoming more complicated and more important every day, and this field of gene transmutation is certain to be intensively investigated. That it will yield much of "practical" importance seems likely; that it may prove a key to the cause of variation and thus of evolution has been seriously suggested. In an environment that we are coming more and more to realize is made up mostly of radiations and vibrations, varying from the ultraviolet to the "cosmic rays" that pierce mountains, it is not impossible that the leisurely-occurring mutations that appear from time to time in nature may be due to a chance hit by some of these all-pervasive rays and waves. Thus "gamma radiation", "milliamperes", "translocation" and a whole

new terminology threatens to be grafted to the Vocabulary of Heredity. If this seems too great a burden, is it not worth while to follow these explorers into a new province of knowledge, and to watch them map and rearrange the inner workings of cell nuclei that are invisible to the naked eye? Exploration is always beset with difficulties and discomfort and those who would follow the explorers, even on the printed page, into so different a world, can hardly hope to escape all the rigors of pioneering.

X-Rays Produce Two Types of Genetic Changes

IT thus appears that two types of genetic modification may be produced in *Drosophila* by the X-radiations—an aberrant distribution of chromosomes and hence new combinations of characters, and gene mutations. In confirmation of these findings in an animal, Stadler reports the production of both types of mutation in plants. In corn, irregular distribution of chromosomes occurs with a certain low frequency in the early cell divisions in endosperm development. This results in mosaic seeds, in which a portion of the endosperm lacks one or more chromosomes. The frequency of occurrence of this phenomenon has been increased about thirty-fold by X-ray treatment at the time of fertilization. Again, in young seedlings of barley, Stadler has produced gene mutations by similar irradiation. In this instance the possibility that the gene mutations may in reality be the result of an irregular segregation has been eliminated. These mutations reported in barley are induced in somatic cells, from each of which a self-fertilizing inflorescence is later derived. Each mutant, therefore, segregates in the progeny of a single head, and its absence in the progeny of other heads of the same plant proves that the chance occurred in the ontogeny of the plants irradiated. Several viable and morphologically distinct recessive types of such gene mutations have occurred in the progeny of the plants thus subjected to irradiation. To these studies by Mavor, Muller, and Stadler may be added the recent work of Gager and Blakeslee upon *Datura*, which shows irregularities of chromosomal behavior as a result of X-irradiation and which suggests gene mutations and other intra-chromosomal changes. The results reported by Goodspeed and Olson upon tobacco plants seem to be in this category, since they do not clearly indicate gene mutations.

From the theoretical standpoint, the chief interest of such results within the field of genetics lies in their bearing upon the problem of the composition and behavior of chromosomes and genes, and thus upon the mechanism of heredity, variation, and evolution. From the practical standpoint, any technique that will induce abundant mutations presents opportunities for the production of improved breeds of domesticated animals and plants beyond anything dreamed of by the slow method of selecting what nature offers. To secure mutations that he desired, Burbank raised plants by the millions, discarding the ninety-and-nines. The advantage of more frequent mutations is obvious. On the medical side, these results, as Muller explains, point to dangers in the practice of temporary sterilization by irradiation. Return of fertility after irradiation does not mean that the germ cells are again normal, since mutations may have been induced that will appear in descendants with disastrous consequences.—CURTISS, W. C. Old Problems and a New Technique. *Science* 67: 141-149. February 10, 1928.

THE EFFECTS OF X-RAYS AND RADIUM

On Species of the Genus Nicotiana*

T. H. GOODSPEED
University of California

FOR over twenty years a collection of *Nicotiana* species has been accumulating in the Botanical Garden of the University of California. Of some 50 species of this genus known from the wild state or in cultivation over one half is represented in this collection. Mendelian studies in a number of polymorphic species, principally of *N. tabacum*, the tobacco of commerce, have been under way for many years along with cytological and genetic investigations of chromosome relations and behavior in a large group of interspecific hybrids.† Through such studies an effort is being made to throw light upon the origins and relationships of the existing species of *Nicotiana* which we know.

During the course of long continued investigation of inheritance in *N. tabacum* here and elsewhere, only a few mutations have been found to occur under controlled conditions. Similarly, changes in plant characters referable to disturbances in chromosome balance are of a very few types, although 24 pairs of chromosomes are involved. With a view to inducing gene mutation and chromosomal irregularities x-ray treatment of sex cells of tobacco was undertaken in 1927. The readiness with which this species responds to such treatment[1] has lead to a variety of studies on the effects of high-frequency radiations on its sporogenous and meristematic tissues. Other species—especially *glutinosa*, *sylvestris*, *rustica*, *Langsdorffii*—are also being looked into from this point of view. A general survey of the results of these studies is included in what follows.

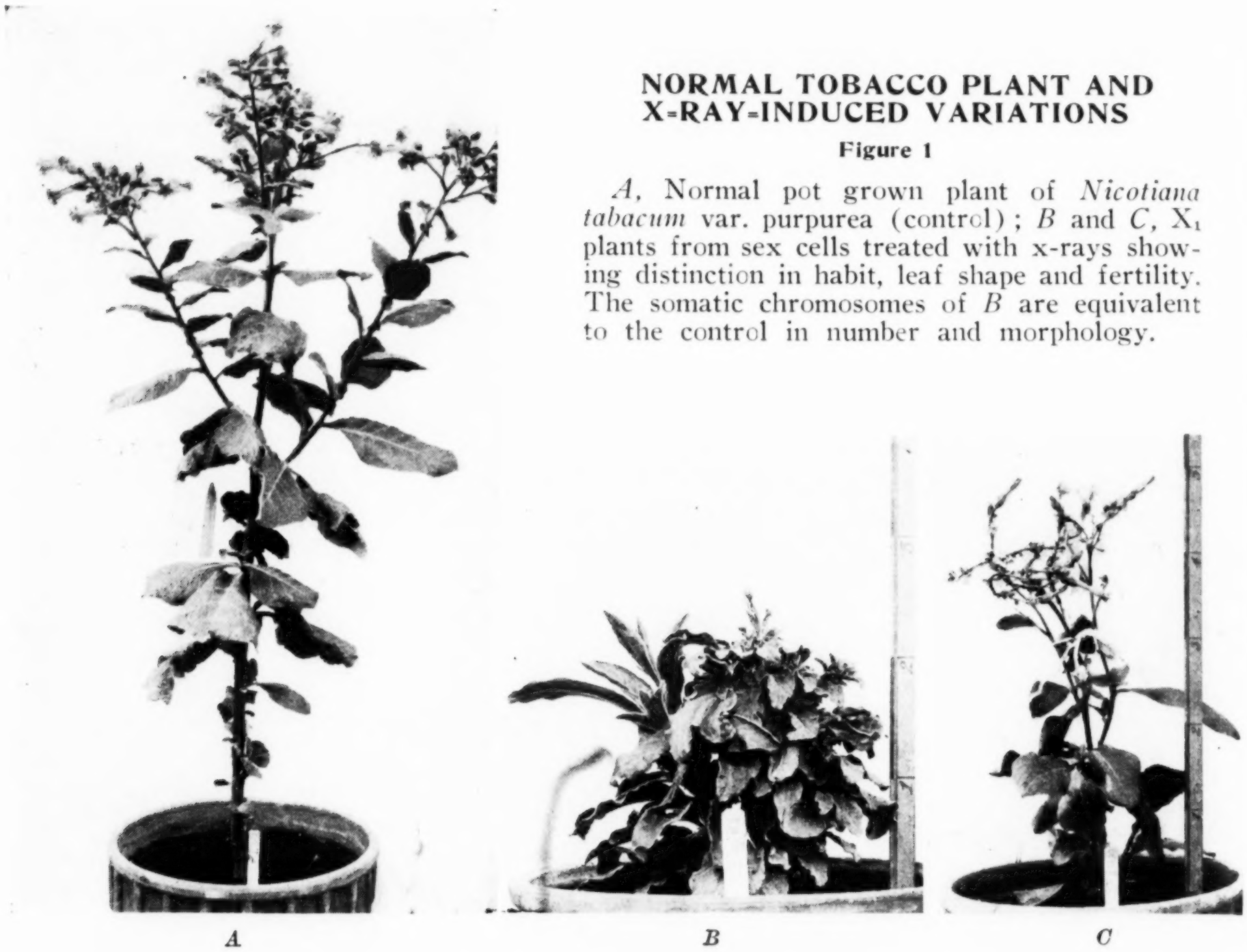
Some account has already been given of the condition of the progeny resulting from the union of x-rayed sex cells of *tabacum*, [1,2,] but no detailed statements have as yet been made as to the nature of corresponding progenies of other *Nicotiana* species or concerning progenies from x-rayed seeds and growing points or as to those from sex cells, seeds and meristematic tissues subjected to the gamma rays of radium. In the following table a portion of the information in hand as to these various combinations is included. The term "variant" refers to an individual which is distinctly different in external morphology from the control. For x-ray exposures a standard type Coolidge tube with air cooled tungsten target was used. The effective potential was 50 KV and the current through the tube was either 2.8 or 5 milliamperes, no filter being used. The times of exposure were various and differed with the tissue concerned. From 12.5 to 100 mg. of radium salt‡ was used with a screen which permitted treatment with gamma rays only.

The distinctions between variant individuals and control were greater or smaller depending, in part, upon the number and character of the plant organs which exhibited alteration. The nature of the changes following x-ray-

*These investigations have been made possible through grants from the Board of Research, University of California, the National Research Council, Division of Biology and Agriculture, Committee on Effects of Radiation on Living Organisms, and through the generosity of Mr. Henry S. Chafee of Providence, R. I., and his family.

†University of California Publications in Botany, vols. 5 and 11 and elsewhere.

‡The radium treatments were made possible by the cooperation of Dr. E. N. Ewer and Drs. W. H. Sargent and R. G. Van Nuys of Oakland, California.



A B C

NORMAL TOBACCO PLANT AND X-RAY-INDUCED VARIATIONS

Figure 1

A, Normal pot grown plant of *Nicotiana tabacum* var. purpurea (control); *B* and *C*, X_1 plants from sex cells treated with x-rays showing distinction in habit, leaf shape and fertility. The somatic chromosomes of *B* are equivalent to the control in number and morphology.

ing of sex cells in the case of *tabacum* has been described elsewhere [2] and are here illustrated in Figs. 1-3 and Frontispiece so far as external morphology is concerned and in Figures 5 and 6 as to cytological details. The visible effects of x-ray treatment of P. M. C.* shown in the latter follow relatively

TABLE I—Nature of first progenies after x-ray and radium treatment of Nicotiana species

For convenience the designations "X" and "R" are used to refer to the progenies produced (1) from the union of sex cells, (2) from seeds, (3) from growing points of seedlings subjected to x-rays (X_1,X_2) or the gamma rays of radium (R_1,R_2).

	Normal	Variant	Total	% Variant
tabacum var. *purpurea*-X_1				
Sex cells	1,775	397	2,172	18
Seeds	143	31	174	18
Seedlings	45	9	54	17
tabacum var. *purpurea*-R_1				
Sex cells	73	21	94	22
Seeds	102	23	125	18
rustica var. *pumila*-X_1				
Sex cells	279	18	297	6
Seedlings	76	17	93	18
sylvestris-X_1				
Sex cells	456	27	483	6
Seeds	36	7	43	16
glutinosa-X_1				
Seeds	84	7	91	8
Seedlings	21	3	24	12
Langsdorffü, var. *grandiflora*-X_1				
Sex cells	187	8	195	4
Seeds	153	3	156	2
Seedlings	19	0	19	0

*Pollen Mother Cells .

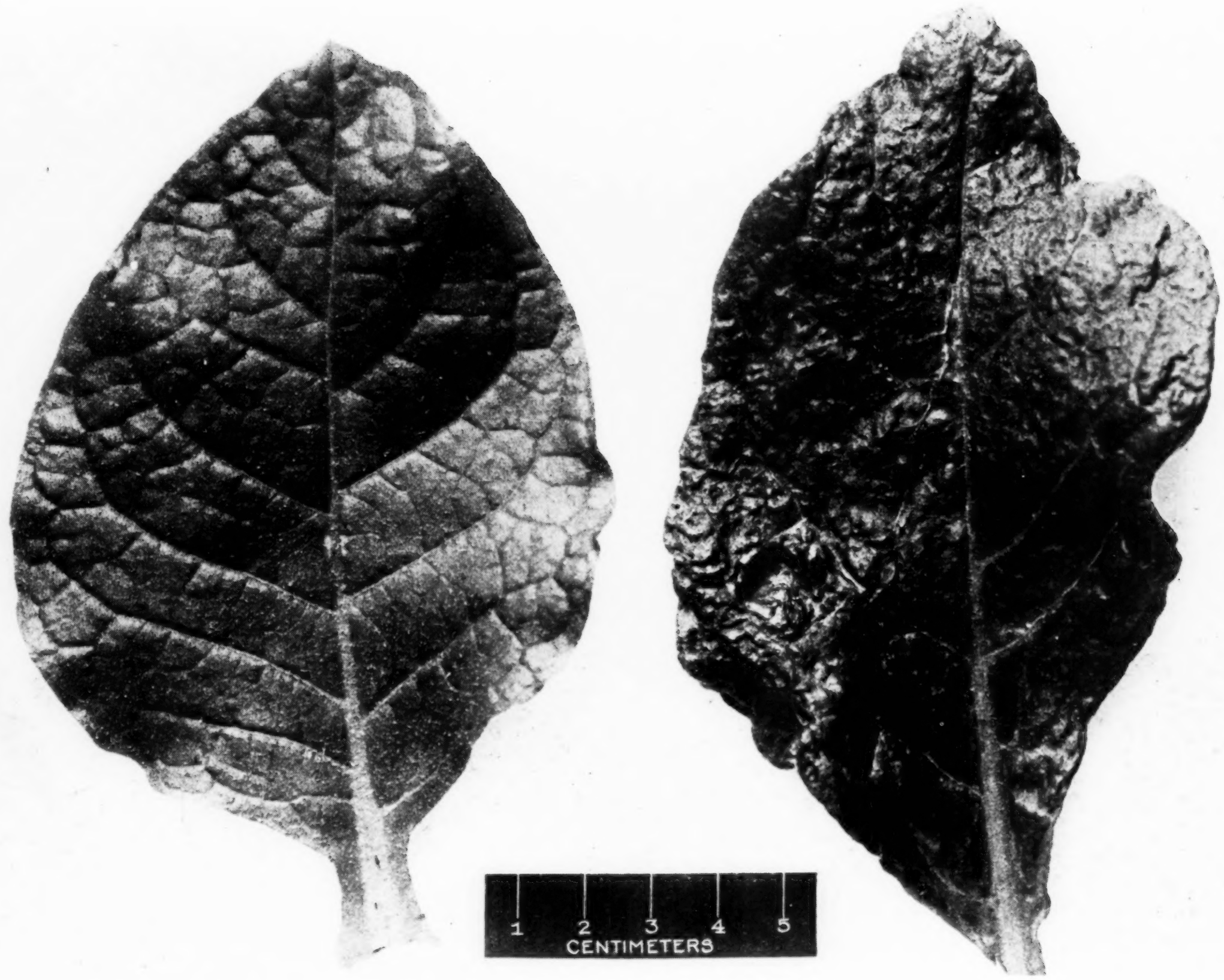
light dosage in the late archesporial stage or early meiotic prophases. As can be seen in Figure 5, non-conjunction, non-disjunction and fragmentation accompany a certain amount of apparently normal conjunction at *I-M** (*e*). The reflection of the chromosomal aberrancies is found at later meiotic stages, the culmination at tetrad formation (Figure 6) giving rise to abnormal sizes and forms of microspores. The same situation follows treatment with the gamma rays of radium.

Chromosome Fragmentation

The continual occurrence of chromosome fragmentation is of interest particularly because the fragments so often become a part of the chromosome garniture of succeeding generations from x-rayed sex cells. A rather unexpected cytological situation is, apparently, involved in the initial fragmentation. It appears that the chromosomes of a pair which were in the early prophases of the heterotypic division when irradiated will usually progress morphologically unchanged throughout the remaining prophases and appear normally formed at the metaphase. When, however, the given P. M. C. has become specific for the anaphasic distribution of chromosomes and when perhaps the chromosome pair has disjoined and the resulting univalents are in the plasma and under the influence of what may be an achromatic figure, then and not until then does fragmentation take place. This situation might be taken to mean that in the chromosome as a result of x-ray bombardment a degree of molecular instability has been created, a disturbance which will express itself in fragmentation only when the chromosome comes under the influence of an energized cytoplasm. The Hartogian hypothesis which pictures the cell in mitosis as a polarized field of force offers an explanation of the situation in question. Molecular arrangements within the chromosome may be so disrupted by irradiation that structural discontinuities follow. Certain of these newly formed internal units may, at the same time, become depolarized. When such an internally but not visibly fragmented chromosome comes under the influence of the anaphasic field of force the polarized areas will separate from the non-polarized and fragmentation will manifest itself. On the other hand, it is not impossible that the chromosome may normally consist of an aggregation of separate units which are closely united by anastomoses or other bonds. Irradiation may serve to break a certain number of these elastic connections. When at anaphase, such a chromosome begins to move to the pole under the influence of the conventionally postulated achromatic figure, the continuities and discontinuities within the modified chromosomes express themselves in visible fragmentation.

Some pollen grains, following x-ray or radium treatment, prove to be viable and after fertilizing untreated egg cells give rise to progeny which include plants whose chromosome garniture is, as might be expected, visibly different from that of the control. We have studied, extensively, the cytological condition of such plants which prove to possess the products of non-conjunction and fragmentation as well as other anomolous chromosomal states. The diaphase shown in Figure 8 (*a, á*) was characteristic of some of the P. M. C. of one highly variant X_1 individual. The total chromosome number is decreased (n = 24 in *tabacum*), the ability to conjugate on the part of at least one pair has been lessened and chromosome fragments, either free or attached, can be seen. This P. M. C. gives a complete reflection of the types of abnormal chromosomal forms and behavior shown in Figures 5 and 6. Peculiarly enough this particular X_1 plant apparently consisted of a mosaic of nuclear elements. The number of chromosomes and fertility varied, at least from one shoot to another, and some P. M. C. appeared to contain almost a normal nuclear volume; chromosome fragments

*1-M=First metaphase.



ABNORMALITY DUE TO RAYING

Figure 2

N. tabacum var. *purpurea*—On the left a leaf from the control; on the right a leaf from an X_1 plant (sex cell treatment) showing extensive abnormality, largely a reflection of tissue inversion and displacement.

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and non-conjunction occurring in all cases, however. As seen in Figure 10 the somatic mitoses of this plant were abnormal and it seems possible that, as a result of initial chromosome unbalance, cell lineages of varying chromatin content were produced by early zygote divisions and persisted, in some cases, to enter into the formation of mature tissues, sporogenous and otherwise.

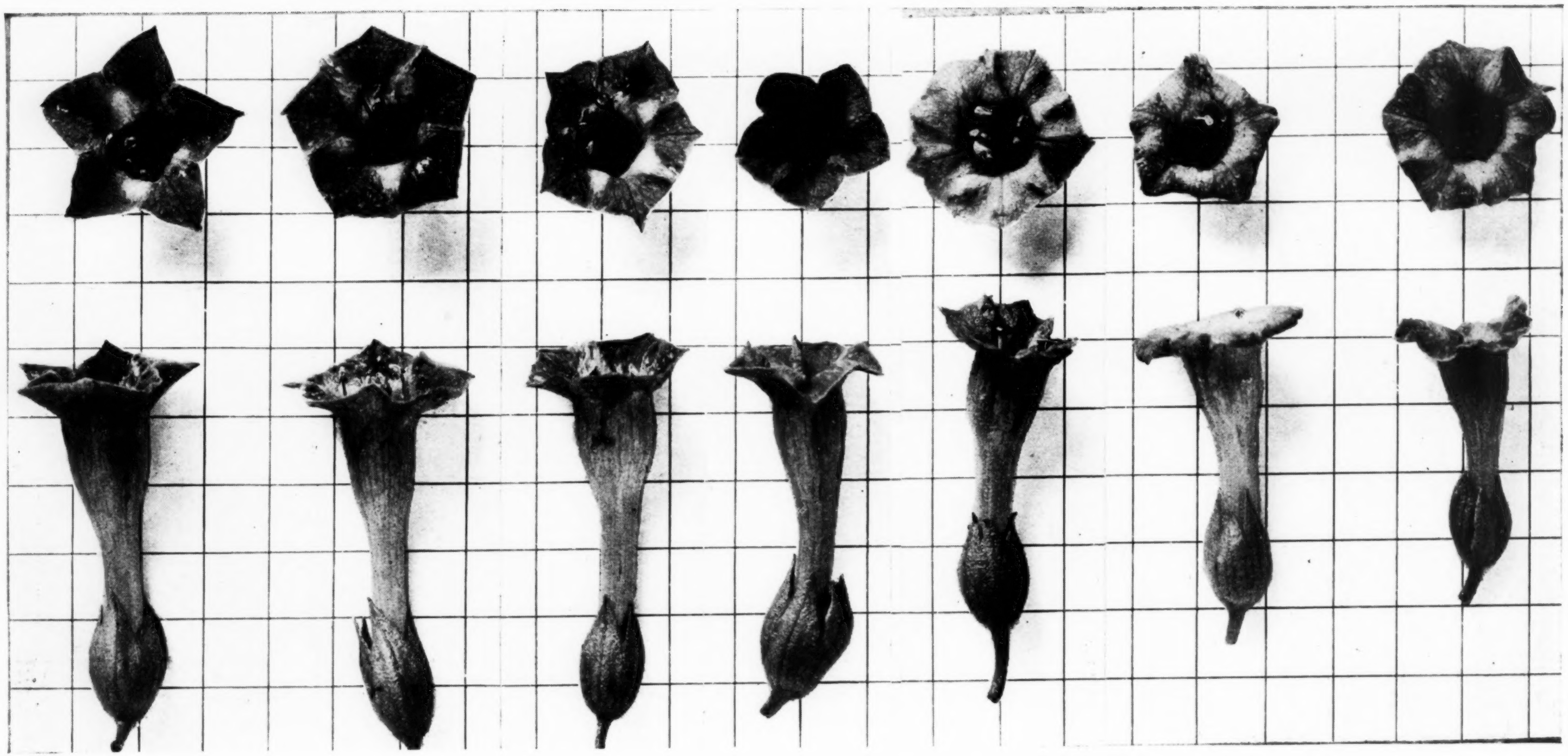
In X_2 50 plants of this variant, selfed, and 50 plants of variant × control were grown. In both populations a recessive gene alteration appeared which was productive of a type of plant possessing 24 $_{II}$ plus chromosome fragments. This situation and the presence of numbers of individuals equivalent to the control indicate that, as was anticipated, only cell lineages approximately normal in chromosome content gave viable combinations.

Some of the distinctions in external morphology in X_1—and equivalent types occur in R_1—are shown in Figures 1-3 for *tabacum* and in Figure 4 for *rustica*. As suggested above, many of these variant types depend upon visible, quantitative chromatin alterations. A considerable number of new monosomic and trisomic races, from non-conjunction or non-disjunction, have resulted and the occurrence of those few which are known to arise spontaneously in the control is greatly increased. A number of highly variant individuals, of which the plant shown in Figure 1, *b* is an example, exhibit no visible chromosomal distinction when compared with the control. They may or may not be completely sterile, as in the plant figured, but uniformly their meioses (X_1) show more or less non-conjunction followed by chromosome lagging. Their progenies (X_2) therefore contain a new series of variant types, whose external morphology is a reflection of quantitative differences in chromatin content in addition, often, to qualitative differences, the latter being responsible for the variant character of the X_1 parent. Gametic or zygotic sterility may also be involved in such cases with the result that the analysis of the genic alterations concerned is difficult and often impracticable.

A great variety of quantitative chromosomal alterations have occurred and some of those investigated may yield evidence of some general cytological significance. The relative unimportance of purely quantitative changes has been apparent, as, for example, in those cases when addition of chromosome fragments to the full diploid complement may be characteristic of a race indistinguishable from the control in external morphology. However, the fact that apparently many X_2 individuals, in one such case at least, contain the fragment, suggests that its presence may be essential for gamete or zygote viability. It has been shown in *N. alata* that a condition of quantitative chromosome unbalance is likely to be accompanied by the production of a considerable number of diploid gametes.[3,4] By analogy, evidence of such

TABLE II—Effects of x-rays on sex cells at different stages of their maturity—Nicotiana Tabacum

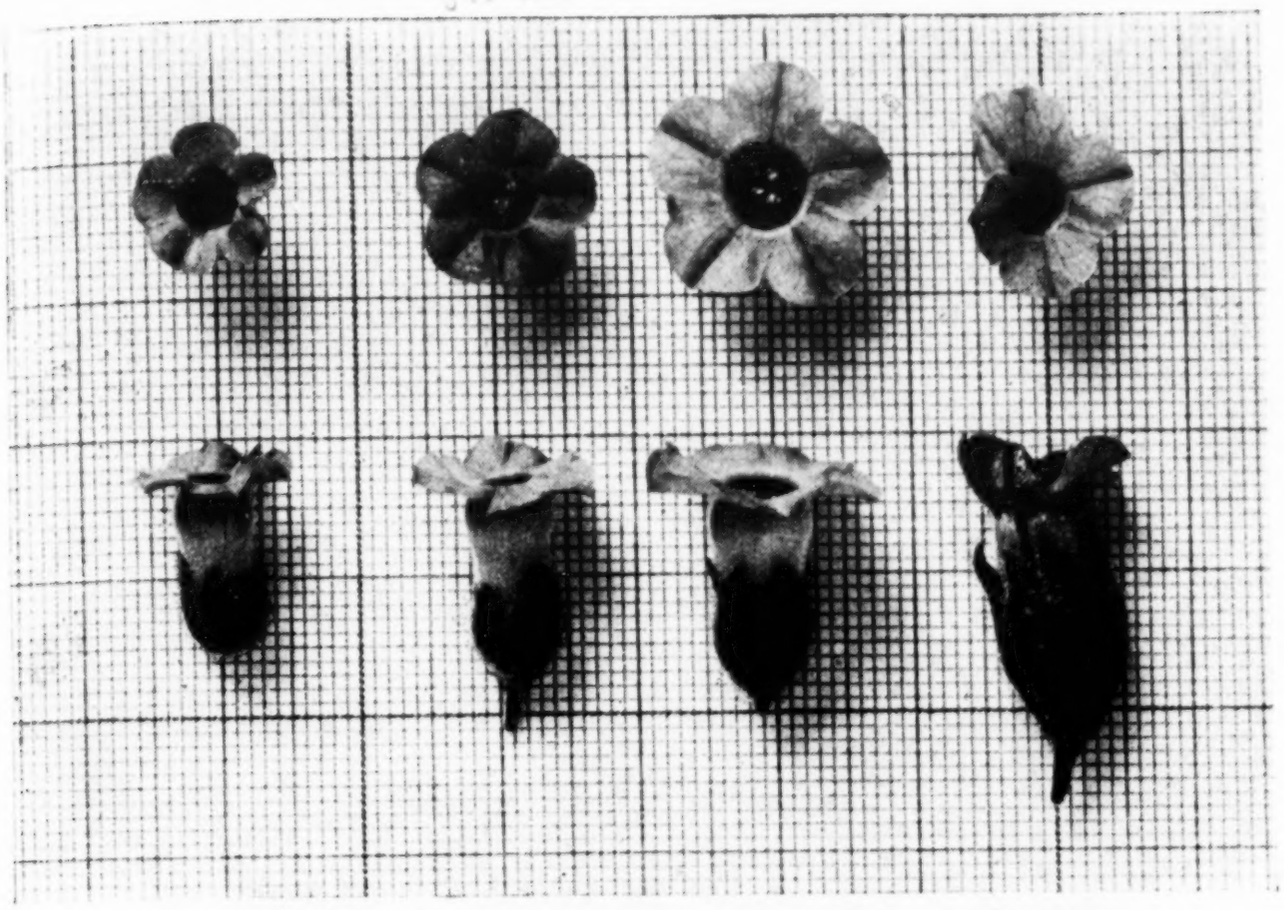
		Normal	Variant	Total	% Variant
	var. purpurea				
I	♂ tetrad ♀ premeiotic	81	16	97	16
II	♂ young pollen ♀ archesporium	131	11	142	8
III	♂ older pollen ♀ meiotic prophases	199	49	248	16
IV	♂ almost mature pollen ♀ I or II-M	83	14	97	14
V	♂ mature pollen ♀ mature embroysacs	118	23	141	16



VARIATION IN FLOWERS

Figure 3

N. tabacum var. *purpurea*—Variation in flower form, size and color displayed in X_1 (from treated sex cells)—the second flower from the left is from the control.



FLOWER VARIATION DUE TO X-RAYS

Figure 4

Variation in size, form and color of X_1 flowers of a yellowish-green flowered species, *N. rustica* var. *pumila*. The second flower from the left is from the control.

chromosomal disturbance should be found in the progenies under discussion. In Figure 9 is shown a I-M plate of a triploid plant which occurred in an X_2 progeny and the occurrence of diploid meioses has repeatedly been noted in studies of X_1 P. M. C. (See Figure 8, *b. c.*).

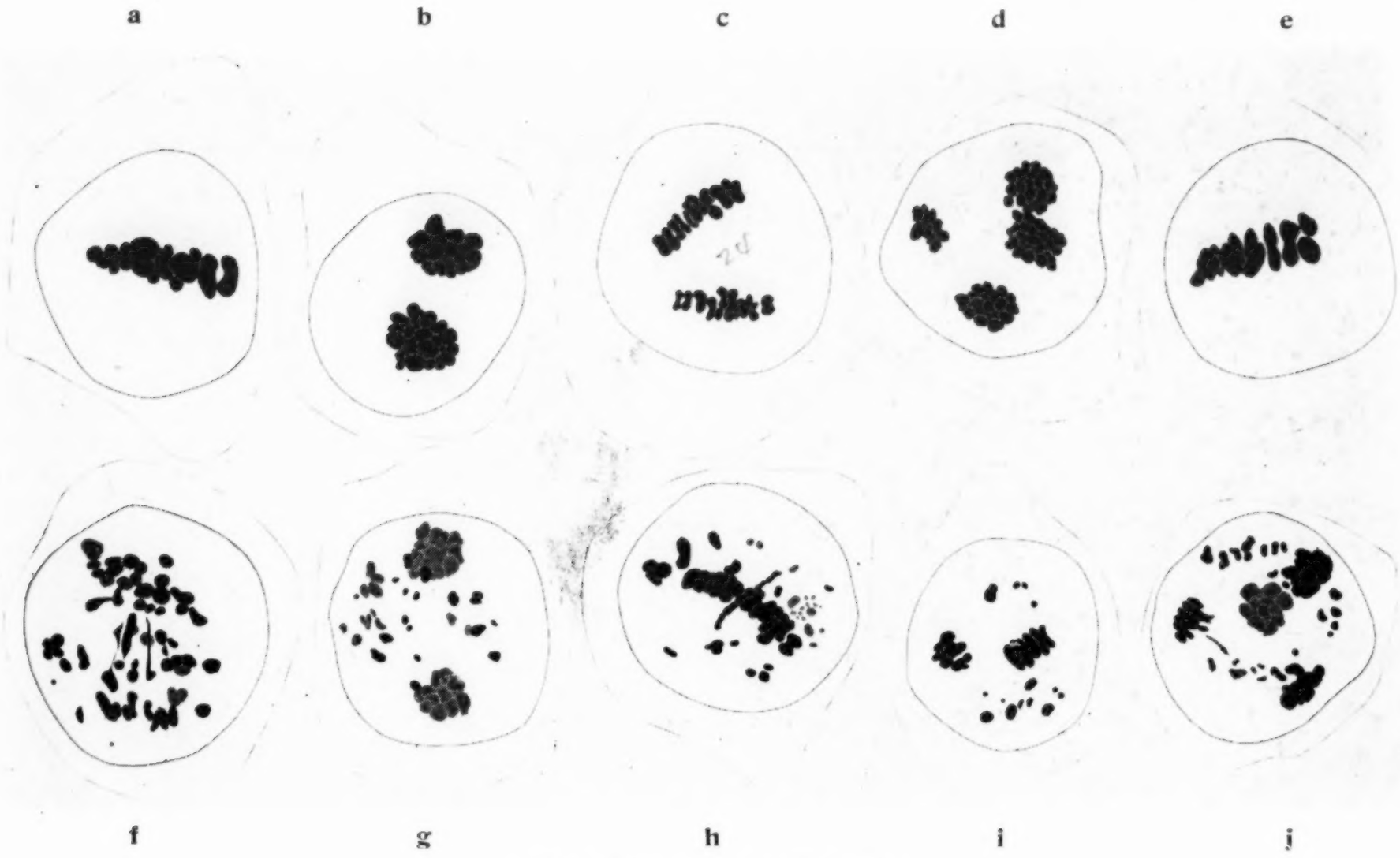
Species Variation in Reaction

Heritable, qualitative alterations of a variety of sorts follow x-ray and radium treatment of sex cells of *tabacum*. Lethality is conspicuous and both dominant and recessive changes in vegetative and floral characters have been found. As indicated above, further genetic analysis in certain of these cases will be complicated. Sex cell treatment of another polymorphic species, *N. rustica* var. *pumila*, is similarly productive of both quantitative and qualitative changes in the hereditary material. By contrast, a considerably greater stability under treatment with high frequency radiations appears to be characteristic either of relatively monotypic species such as *nudicaulis* (n = 24) or *sylvestris* (n = 12) or, in general, of species with lower chromosome numbers; for example, *Langsdorffii* (n = 9) and *glutinosa* (n= 12). However, as indicated in Table I, treatment of sex cells of *sylvestris* did produce some variants in X_1 and the same is true of *Langsdorffii* var. *grandiflora*. An analysis of second generation progenies in the case of certain of these species has not been carried out.

Some preliminary evidence concerning the relation of stage of maturity of sex cells to the production of variants in X_1 under constant dosage is included in Table II.

There is an indication in these figures that stage of maturity has little relation to effectiveness of x-radiation. The fact that the maximum percentage of variant occurrence followed treatment at a time when one of the sex cells was in prophases of meiosis is probably significant, however. Obviously, these experiments were not devised in a fashion most favorable for the securing of the desired information and they are being repeated.

Although it is clearly more scientific to study radiation effects where single cells—sex cells—are treated, interest also attaches to the effects of treatment of growing points. As will appear in what follows, treatment of germinating seeds or young seedlings with x-rays or radium may give rise to mature plants which represent a mosaic of nuclear elements reflected in differences in character expression and particularly in fertility on different parts of the same plant. Practical utilization of this type of effect should be possible whenever vegetative propagation of a favorable somatic reflection of altered nuclear conditions is possible. This pos-



ABNORMAL DIVISION

Figure 5

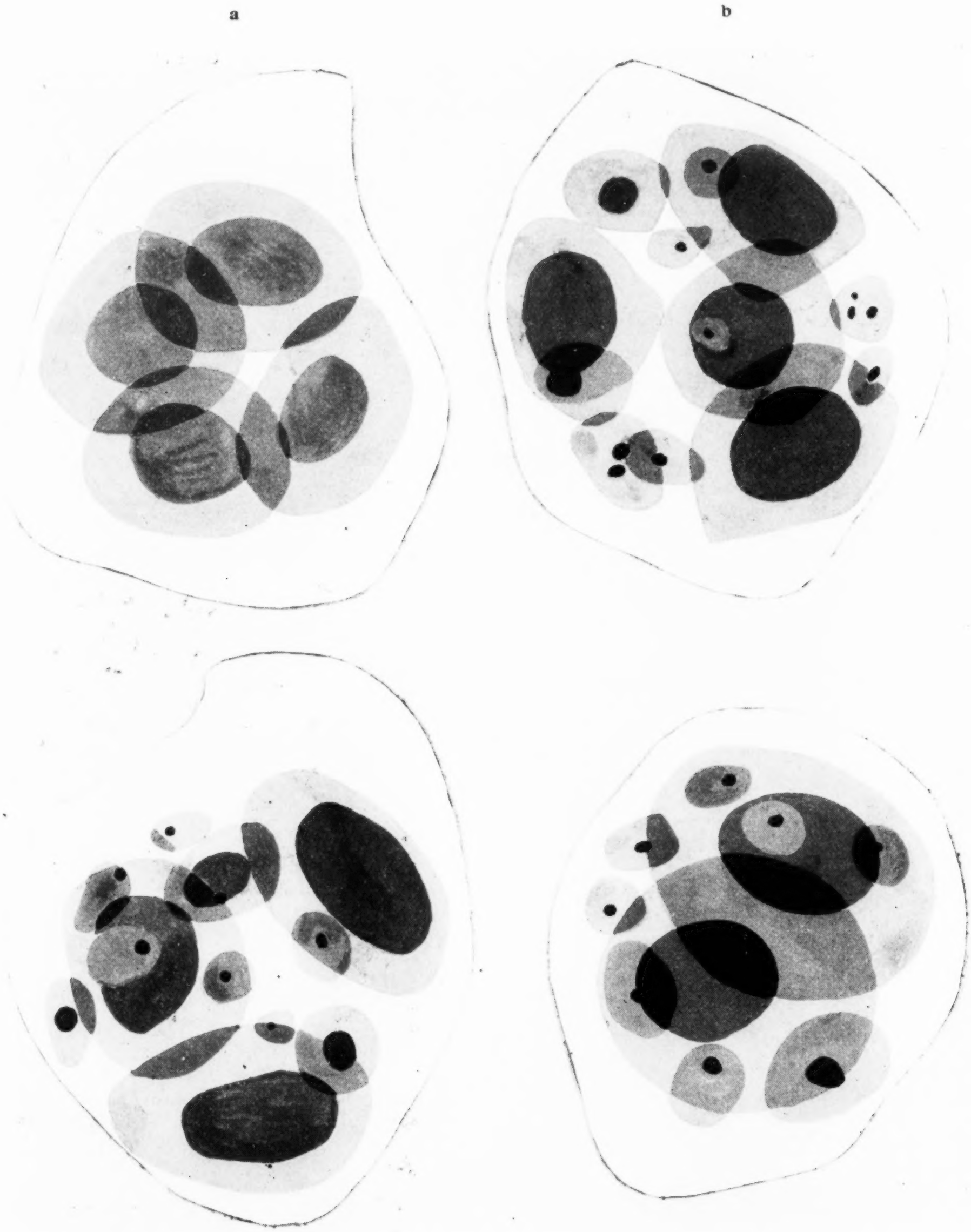
N. tabacum var. *purpurea*—P. M. C. (Pollen Mother Cells), *a-d* from the control: *e-j* after x-raying in early prophase. *a* and *e* = 1st metaphase, *f* = 1st anaphase, *b* and *g* = 1st telophase, *c*, *h* and *i* = 2nd metaphase, *d* and *j* = 2nd telophase. In *e-j* are shown a variety of abnormal meiotic conditions, the result of x-radiation—chromosome fragmentation, non-conjunction, non-disjunction, and lagging of chromosomes. In *h* the two 2nd metaphase plates have fused, quite a common phenomenon.

sibility is appreciated by various horticulturists who have recently been subjecting meristematic tissues of perennial species to x-rays.

Resistance of Seeds and Susceptibility of Seedlings to X-Rays

As contrasted with maturing sex cells, mature pollen and ungerminated seeds are, of course, highly resistent to effects of high frequency radiation and, indeed, it is difficult to find a lethal dosage for seed of *Nicotiana*, and the same is apparently true for other genera also. It seemed desirable to secure an x-ray dosage which would prove lethal to a large proportion of the seed. Such dosage would then be taken to represent the maximum effect upon the hereditary material and working backward from it there seemed to be a possibility of relating dosage to incidence of heritable and other alteration. However, in experiments involving over 15,000 seeds with shorter and longer wave length for long periods of continuous or intermittent exposure no lethal effect has been observed. For the gamma rays of radium, such effect occurs only after 40 hours continuous treatment with 50 mg.; the salt and seeds being in contact with the filter.

The absence of lethal effects after x-ray treatment is illustrated in Figure 7. The treated seed (at left) was exposed continuously for three hours to 50 KV. and 5 milliamperes at a distance of 20 cm. without filter. At the end of three weeks total germination was over 95 per cent in both treated and control. However, the initial rate of germination is clearly retarded and at the end of the period shown this retardation roughly corresponds to the distinction in development between the 14 and 21 day controls. It is mainly a transient effect, however, and so far as size and vigor at maturity are con-



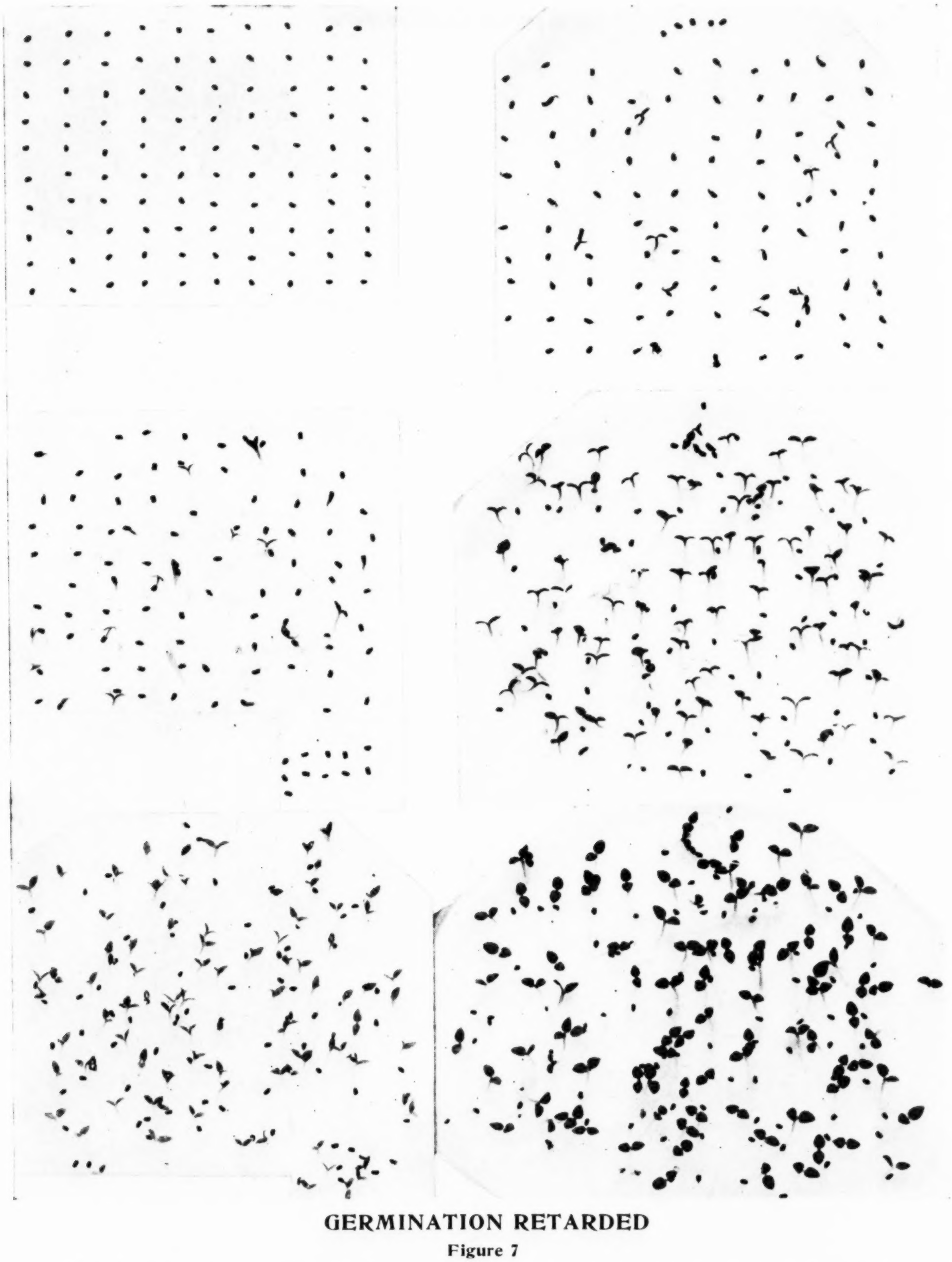
ABNORMAL POLLEN FORMATION

Figure 6

N. tabacum var. *purpurea*—tetrads of microspores. *a*, from control P. M. C., *b-d* typical products of the abnormal meiotic conditions shown in Figure 5, *e-j*.

cerned no general effect of x-radiation can be seen.

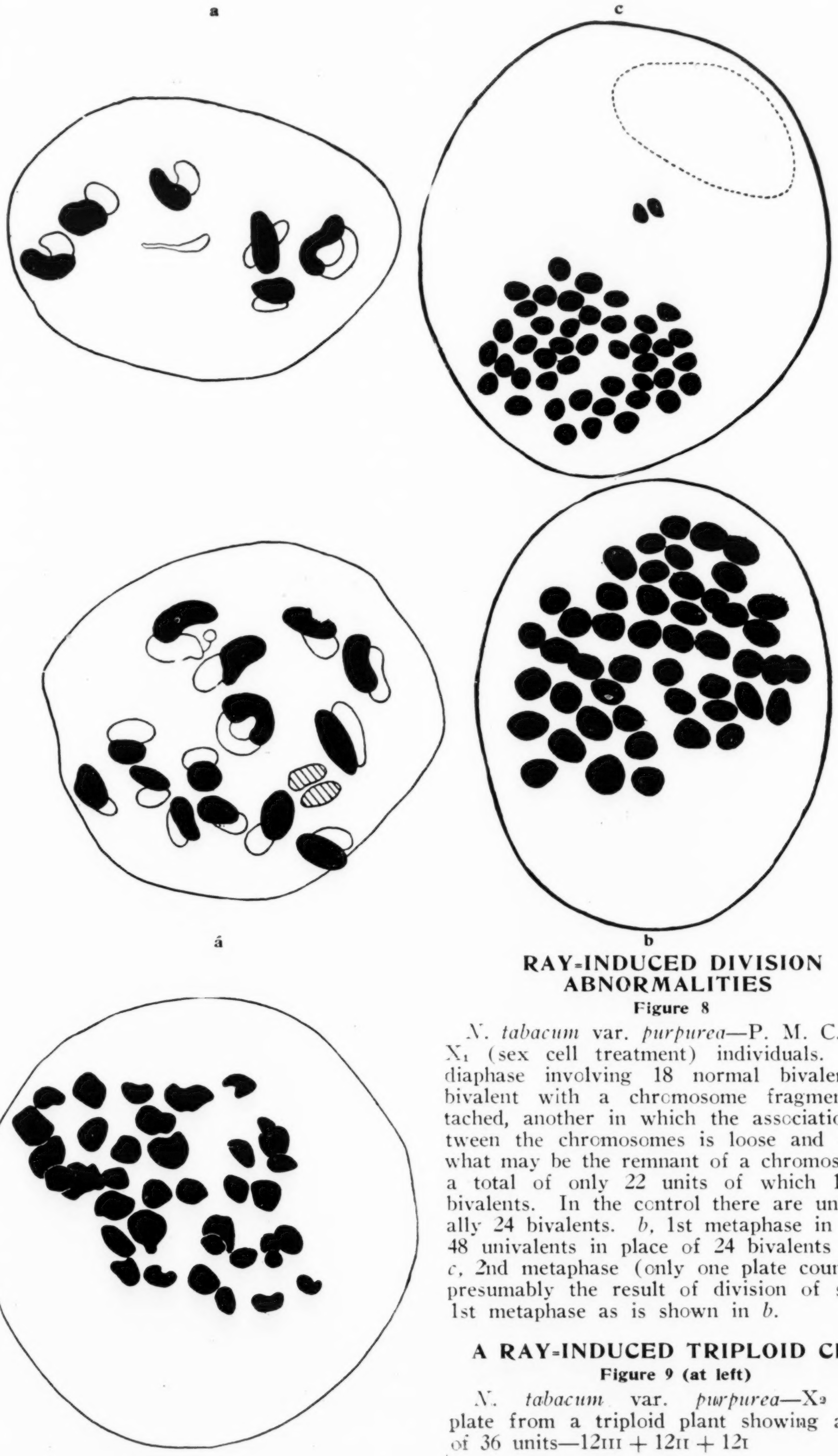
On the other hand, when germinating seed or seedlings in the cotyledon stage are x-rayed there is no difficulty in producing lethal effects and those plants which survive exhibit a permanent as well as a transient distinction in growth and form. This situation is illustrated in Figures 11-15. Seedlings



GERMINATION RETARDED

Figure 7

N. tabacum var. *purpurea*—influence of x-radiation upon germination of dry seeds. At right the control and at left the treated seeds, 7, 14, and 21 days after treatment. No lethal effect has occurred following x-raying but early growth has been retarded.



RAY-INDUCED DIVISION ABNORMALITIES

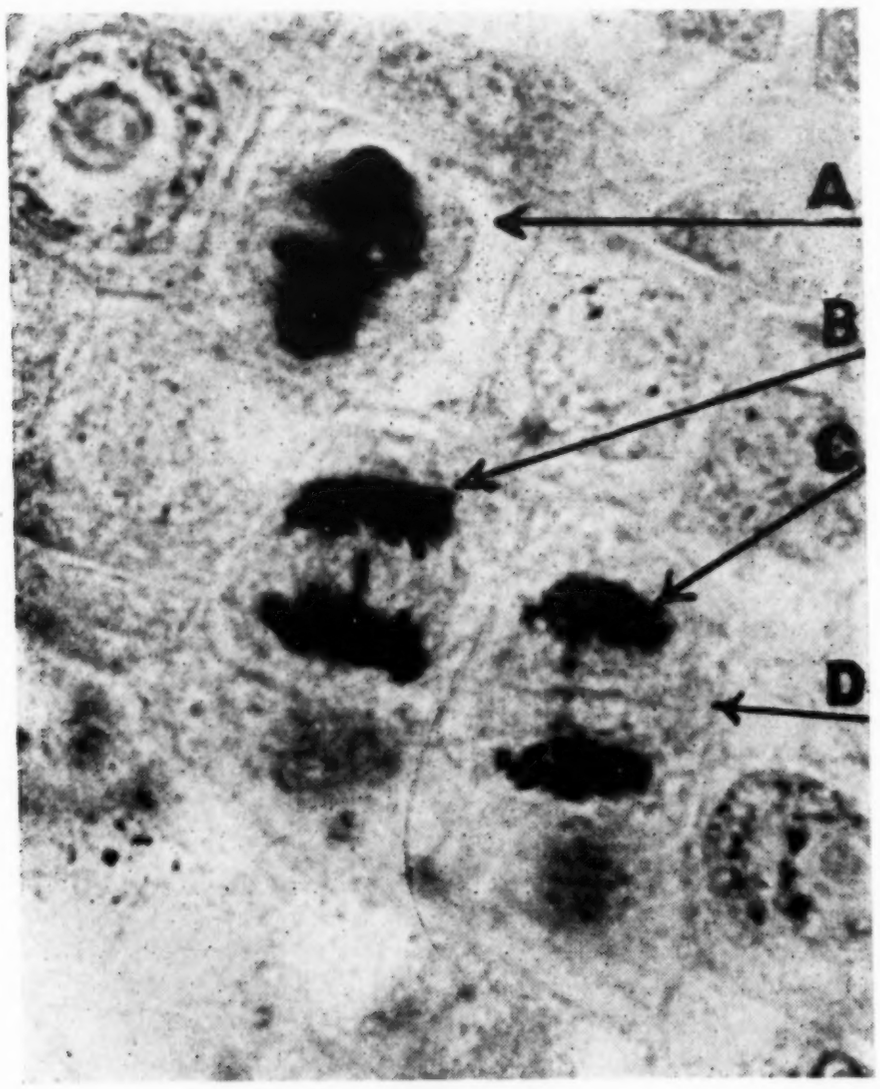
Figure 8

N. tabacum var. *purpurea*—P. M. C. from X_1 (sex cell treatment) individuals. *a*, *á* diaphase involving 18 normal bivalents, a bivalent with a chromosome fragment attached, another in which the association between the chromosomes is loose and finally what may be the remnant of a chromosome—a total of only 22 units of which 19 are bivalents. In the control there are uniformally 24 bivalents. *b*, 1st metaphase in which 48 univalents in place of 24 bivalents occur. *c*, 2nd metaphase (only one plate countable) presumably the result of division of such a 1st metaphase as is shown in *b*.

A RAY-INDUCED TRIPLOID CELL

Figure 9 (at left)

N. tabacum var. *purpurea*—X_2 I-M plate from a triploid plant showing a total of 36 units—$12_{III} + 12_{II} + 12_{I}$



ABNORMAL MITOSES

Figure 10

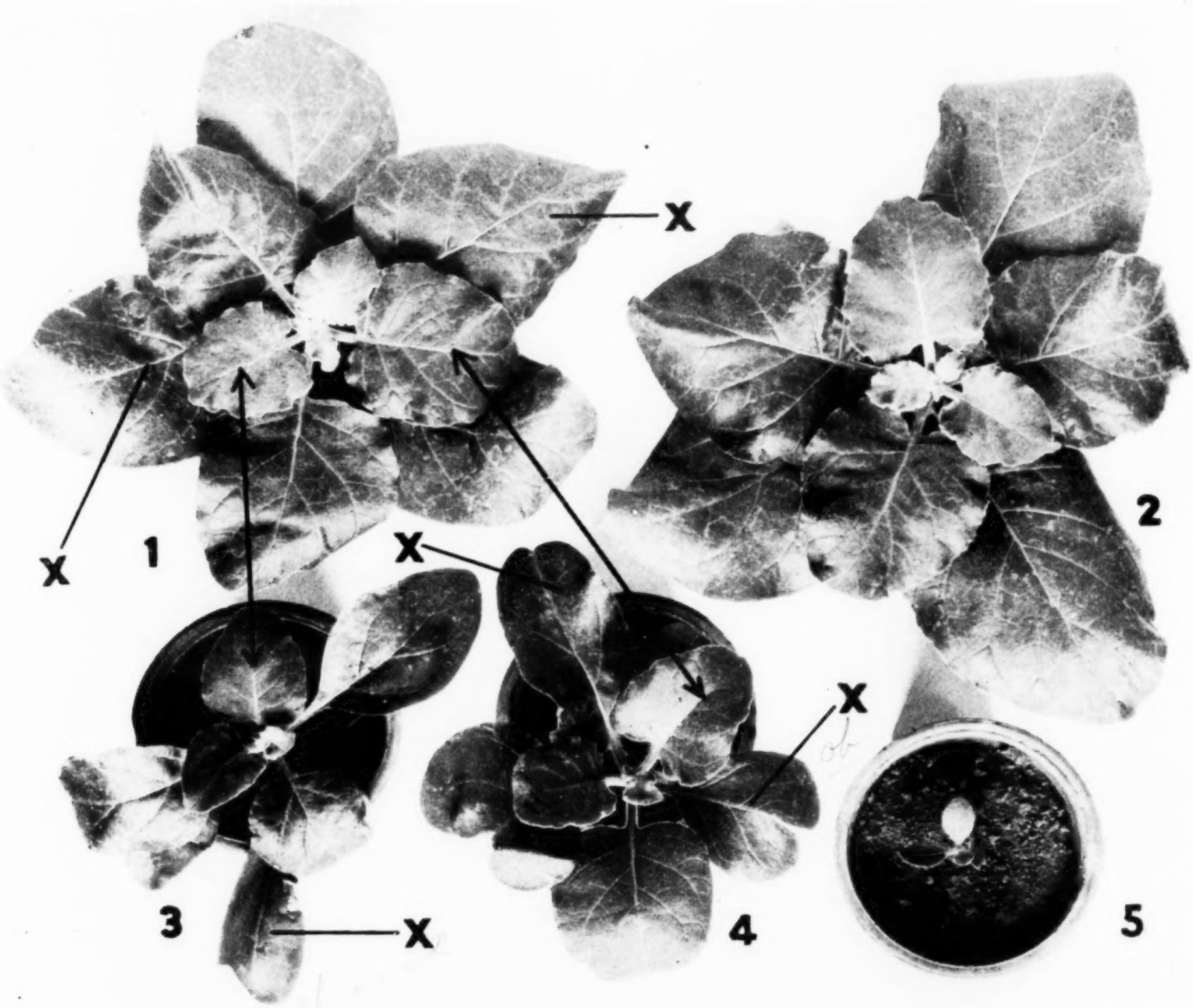
N. tabacum var. *purpurea*—Photomicrograph of longitudinal section of roottip from X_1 plant (sex cell treatment) showing abnormalities in mitosis. *A*, early anaphase with long twisted chromosome in center of the plate; *B*, early telophase with chromatin in plasma; *C*, late telophase, the cell plate *D*, dividing the chromatin strand. (A diaphase from this same plant is shown in Fig. 8.)

of *N. paniculata* in the cotyledon stage, some three weeks old, were x-rayed and the photographs reproduced in Figures 11, and 12 were taken six weeks later. As shown in Figure 11 more or less permanent alterations have been produced. In some cases (Figure 12) the normal appearance of the young plant may be the result of the replacement of an abnormal by a normal axis following the proliferation of an apparently unaffected lateral bud. The mortality after treatment, of seeds which have just broken the seed coat, is very high and the plants which survive are usually abnormal throughout or obviously reflect a mosaic of nuclear elements within. These points are illustrated in Figures 13-16. Typical distinctions in growth rate, habit, leaf form, and in fertility between treated and control can be seen in Figures 13 and 16 in the case of *N. rustica* var. *pumila*. The same types of effect follow equivalent treatment of germinating seeds of *tabacum*.

Some attention has been given to the histological situation in normal and abnormal organs produced by such plants. In the first place, it is clear that mitotic irregularities, comparable to those shown in Figure 10, are occurring. In particular tissues or throughout the soma they may continue or the cell lineages which they produce may fail and be replaced by others more normal in nuclear content and behavior. Displacement and replacement of tissues may, thus, be going on constantly to produce such a situation as is shown in Figures 2 and 14. Cross sections of leaves of a plant from germinating seed which was x-rayed exhibit various structural pecularities. Some interest attaches to the situation illustrated in Figure 16. Cell number, form and arrangement is the same in treated and control but increase in cell volumes in the former accounts for the abnormal leaf thickness. On the other hand, so far as could be determined, there was no distinction in nuclear volume. The nucleo-cytoplasmic ratio has apparently been altered, perhaps as a result of some induced inhibition of mitosis without decrease in the growth capacity of the protoplast.

Genetic Changes in Rayed Seeds

Although treatment in the case of dry or soaked seeds had neither an initially lethal effect nor any appreciable influence on growth rate, height, or weight at maturity, still it may result in significant cytological and genetic alterations. In progenies which gave no evidence of physiological modification, we have obtained variation in fertility, in details of leaf form, and in flower color comparable in character and in behavior to some of the variations obtained in progenies from x-rayed sex cells. This situation has a number of implications. In the first

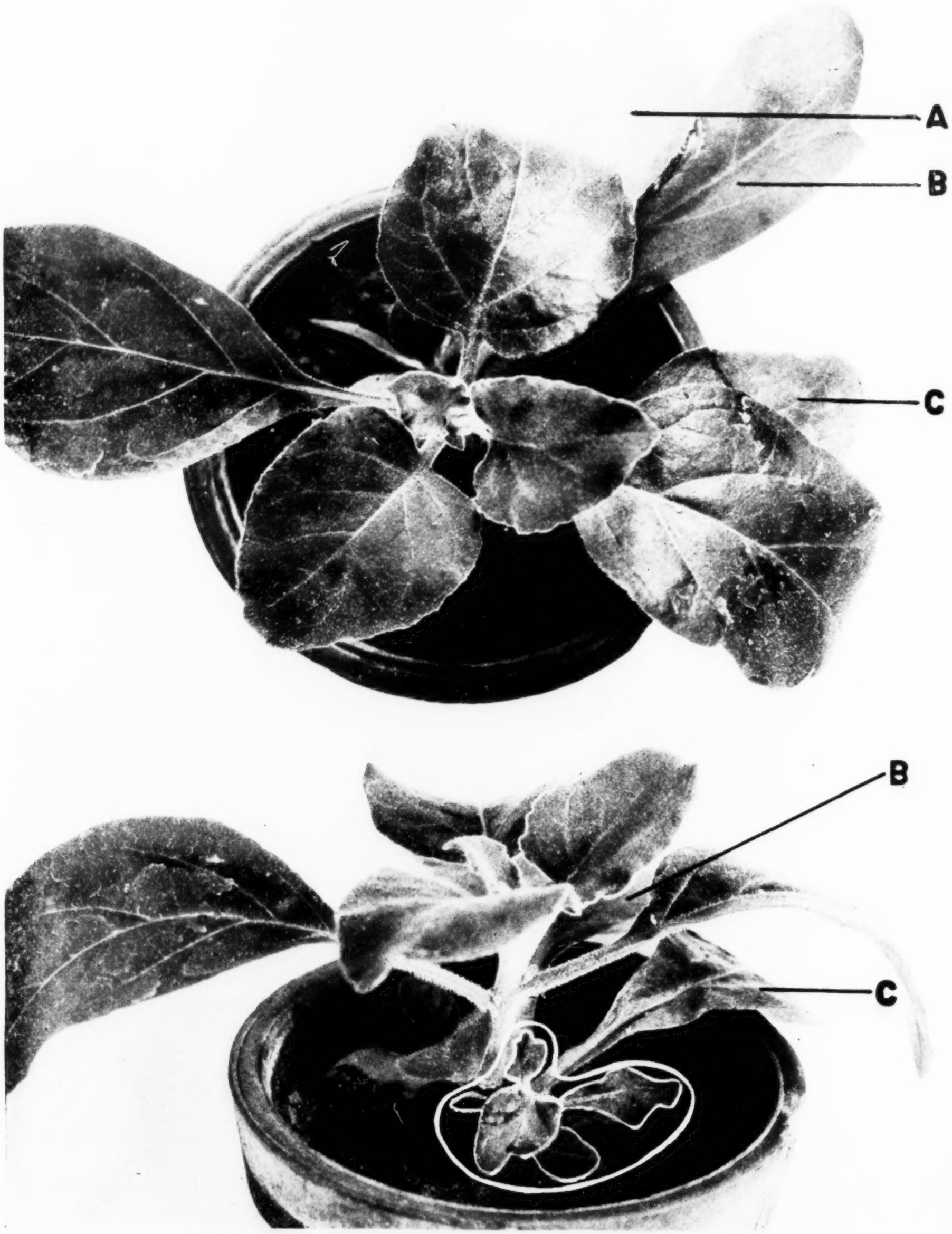


VARIATION IN CHARACTER AND RATE OF GROWTH

Figure 11

N. paniculata—young plants; 1, of control; 2-5, from x-rayed seedlings. Plants 3 and 4 produced, first abnormal leaves ("X," comparable leaves of control also marked "X") but later more nearly normal ones (connected by double arrows with comparable leaves of control). Plant 2 identical from the start with the control; plant 5 highly abnormal and very slow growing.

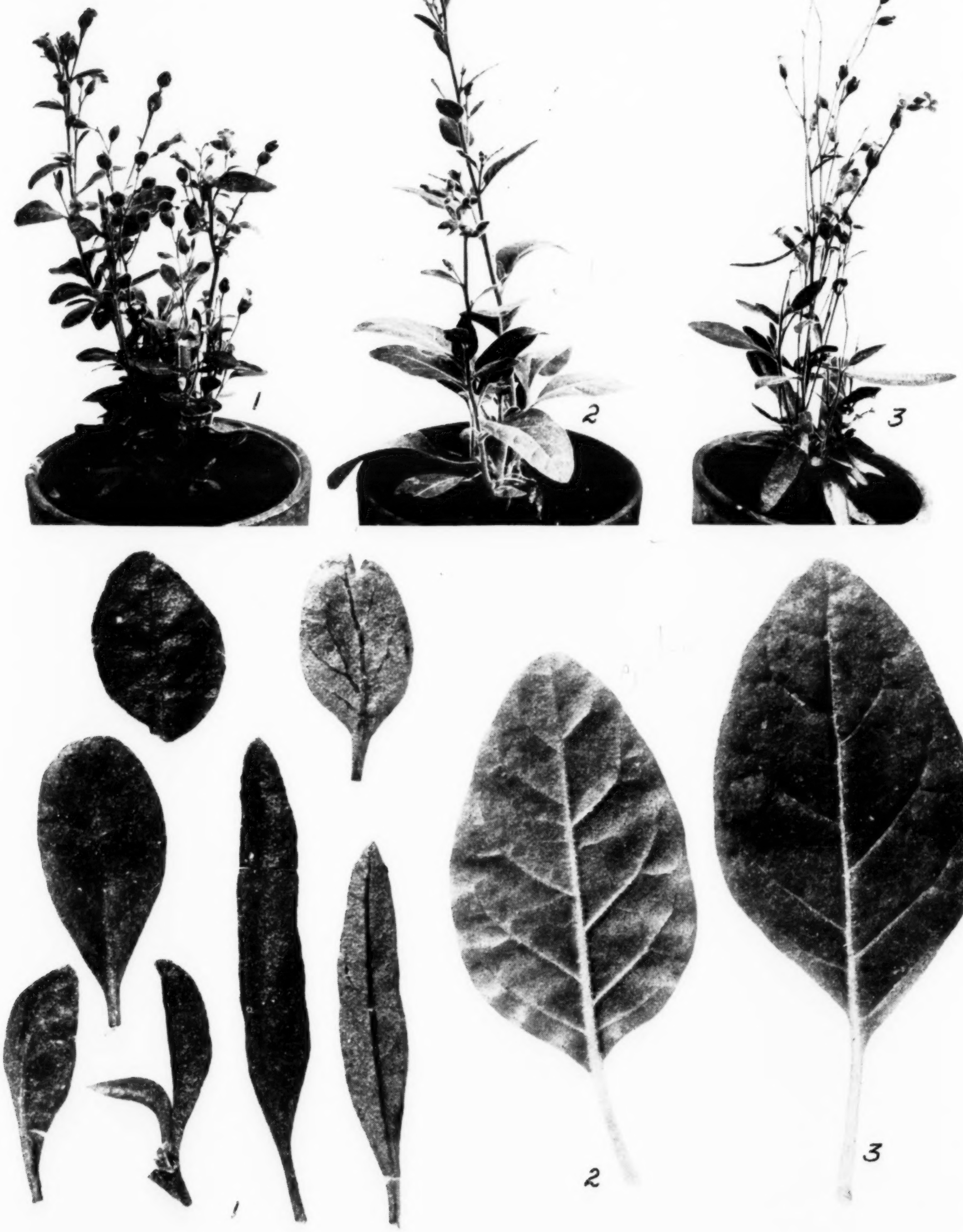
place, on the plant side alone, a very considerable literature may be consulted which deals with the physiological consequences of irradiation of non-sporogenous tissue. These experiments have been in progress for many years. In the light of the results just described and those of others, it seems possible that if certain at least of these physiological investigations had been conducted with even a minimum of genetic imagination the discoveries of the last two years with which Muller's name will always be associated, might have been anticipated. Not only this, but it also appears that certain of the physiological responses reported in these experiments should be assigned to genetic or cytological effects of the type which we have just been describing—in other words, to effects which might not be taken into account by the physiologist. Obviously, the initial genetic constitution of the material, a point which the physiologist understands that he must take into consideration, will play a much more prominent rôle in experiments on the effects of x-radiation than in certain other types of physiological experimentation.



NORMAL DEVELOPMENT FROM LATERAL BUD

Figure 12

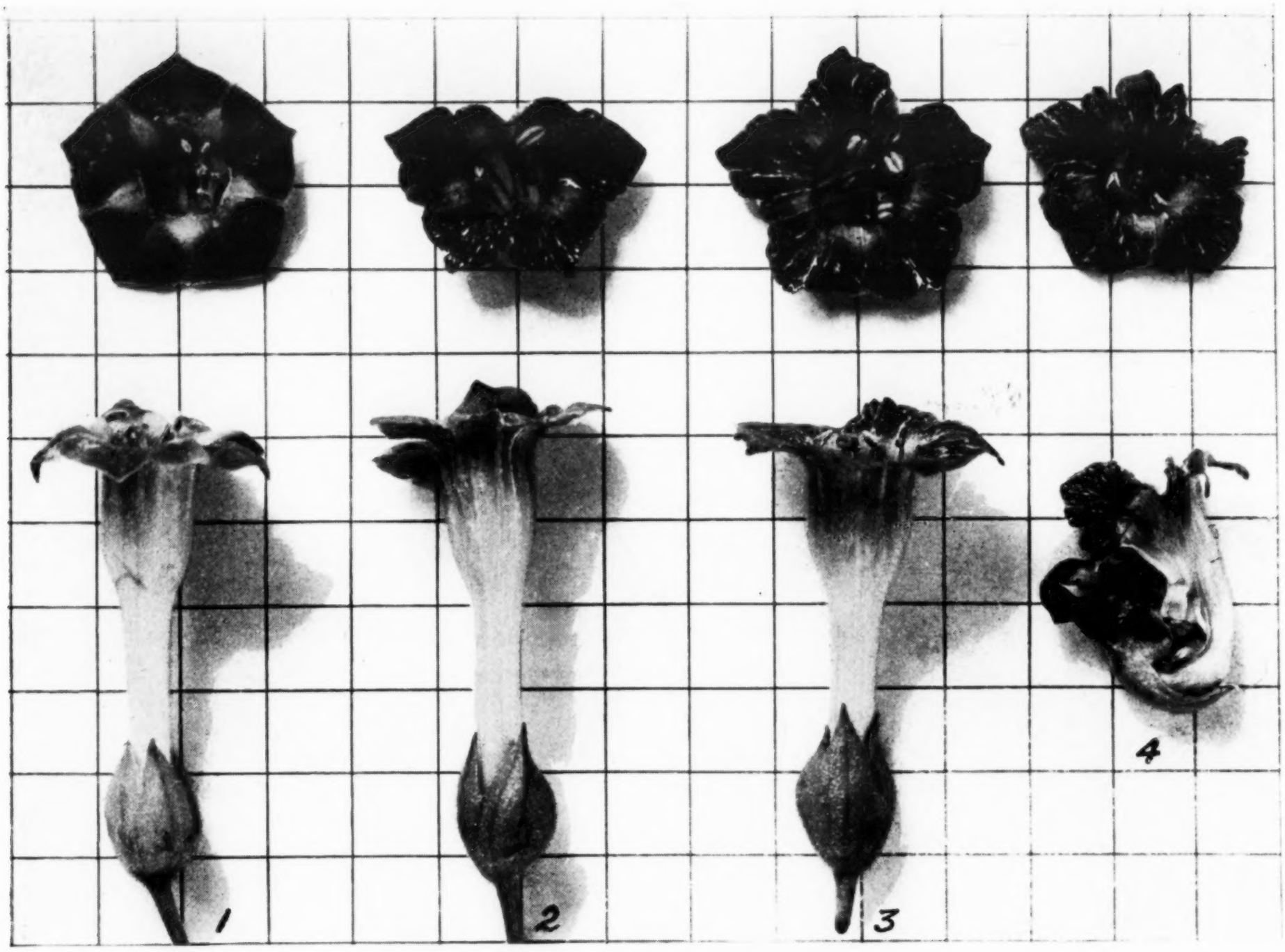
N. paniculata—view from above (upper) and from the side (lower) of plant 3 in Figure 11. The first leaves (within white line) from the original apical growing point were few and abnormal. A lateral bud, apparently unaffected, thereupon developed and from it the mature plant, normal in appearance, will be built.



ALTERED GROWTH RATES

Figure 13

N. rustica var. *pumila*—plant *2* is the control, long past its first blooming and cut back; plants 1 and 3, from seed x-rayed after germination was under way, show distinctions in growth rate in flower form, leaf shape and fertility on different shoots. Leaves *2* and 3 from the control, leaves 1 from plants 1 and 3 above.



MOSAICS OF AIR-FILLED CELLS

Figure 14

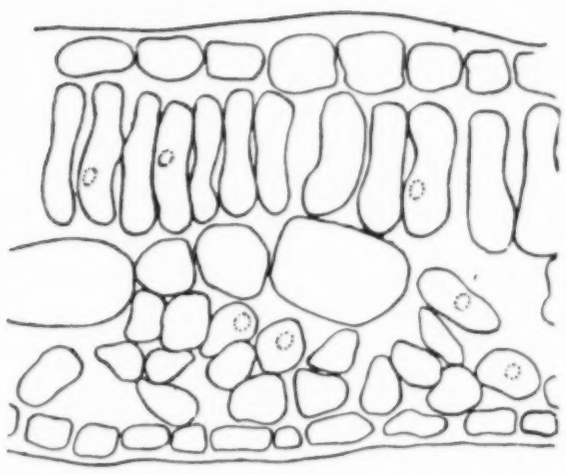
N. tabacum var. *purpurea*—1, a flower from the control; 2-4, flowers from an x-ray plant showing effect of displacement and replacement of tissues—the white dots and lines represent areas of air filled cells.

CHIMERAL EFFECTS IN FORM AND FERTILITY

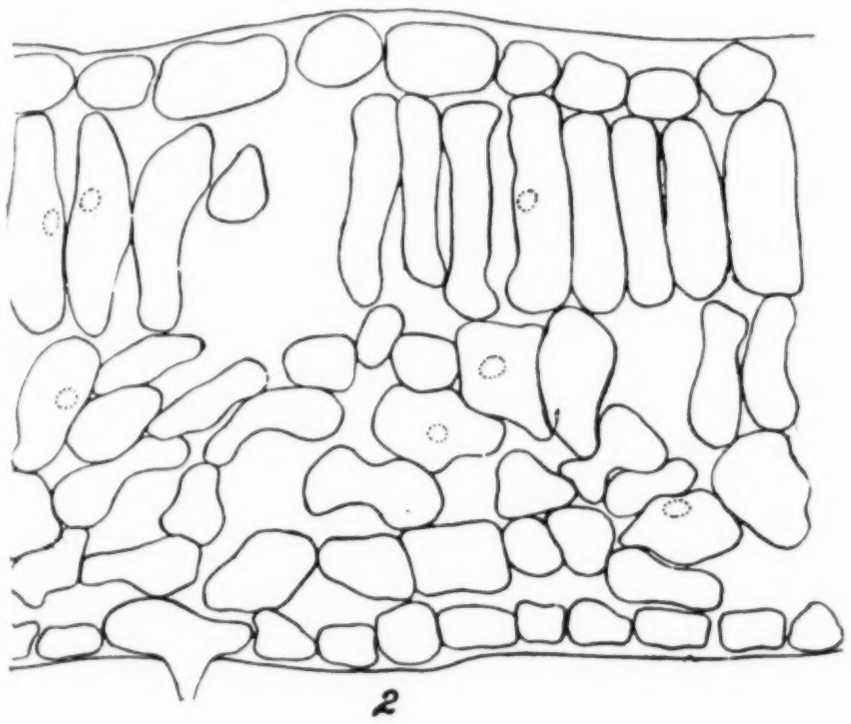
Figure 15

N. rustica var. *pumila* — distinctions in flower form and fertility on different shoots of plant 1 of Figure 13.

In the second place, the question arises as to why x-rays acting on dry, or soaked but not germinating seed should produce gene mutations and chromosomal reorganizations and no appreciable physio-



1



2

INCREASE IN CELL VOLUME

Figure 16

N. rustica var. *pumila*—cross sections of leaves, 1 control, 2 from plant 1, Figure 13. In 2 number and arrangement of cells corresponds to control but cell volume is much increased with no parallel increase in nuclear volume.

logical response, whereas the same wave lengths acting upon germinating seeds produce these same genic and cytological changes, and, in addition, prove lethal to a certain proportion of the seed and in the remainder are followed by marked changes in growth and form both externally and internally expressed. If the nucleus is the dynamic center of the cell, with administrative functions which determine or at least direct all cell activities and thus all morpho-genetic processes, it might be anticipated that any vital nuclear alteration should be reflected in cessation of cell activity or in abnormalities of growth response. This does not appear to be the case, at least if one agrees that changes in the hereditary material, apparently of evolutionary significance, are vital changes.

This situation might suggest that the growth substratum is not within the nuclear sphere of influence. It would, however, seem more appropriate in this connection to disregard any postulated distinction between nucleus and cytoplasm. Growth might be tnought of as the product of reaction between a number of protoplasmic units or factors—and that in particular a proper or favorable juxtaposition of these factors is necessary before any reaction can occur. A somewhat crude analogy might be drawn from the behavior of hydrogen and chlorine under the influence of different pressures. In the dry seed these favorable juxtapositions cannot take place, since they are determined by the environmental conditions recognized as essential for germination. When these conditions are realized, a favorable juxtaposition occurs and is followed by germination and later evidences of growth. X-rays would, thus, prove lethal to *germinating* seeds by altering all or most of the favorable juxtapositions which had taken place or they would produce changes in growth and form when a lesser number are altered. Heavy dosages which produce a transient effect upon germination and early growth, cause a displacement which is not permanent—molecular disturbances rather than rearrangements—permitting the ultimate reassumption of favorable juxtapositions, the proper environmental conditions still obtaining. Finally, a dosage lethal for seeds that have not germinated may require the actual destruction of all or most of the individual growth units, an exponential or probability relation being involved.

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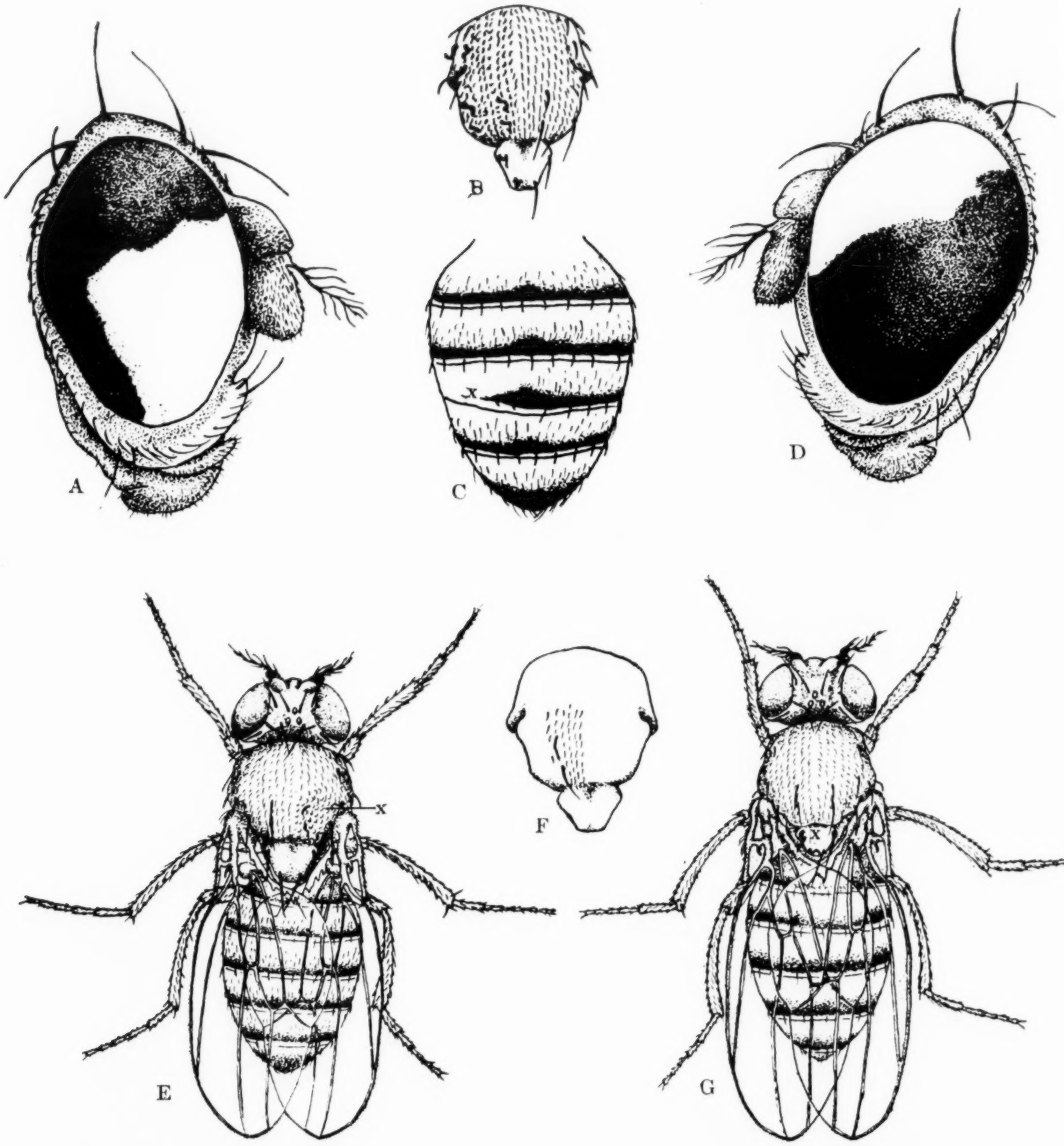

EYE AND BRISTLE SOMATIC MUTATIONS INDUCED BY X-RAYS

Figure 17

A—Right eye of male fly showing approximately one-half of the eye white, the result of a gene mutation. From a culture of eggs rayed at seven to eight hours of age. *B*—The thorax of a female fly showing the left half "singed." From a culture of eggs rayed at one to seven hours. *C*—A spineless area on the abdomen (at x). From a culture of eggs rayed at one to ten hours. *D*— Left eye of a male fly showing about one-third of the eye has mutated from red to white. From a culture of eggs rayed at fourteen to sixteen hours. *E*—A female fly showing (at x) a singed area consisting of four large bristles and about forty hairs. From a culture of eggs rayed at six to eighteen hours. *F*—Dorsal view of a thorax showing a yellow area consisting of two large bristles and about forty hairs. The gray bristles and hairs have been omitted. From a culture of eggs rayed at seven hours. *G*—Female fly showing the two left scutellar bristles singed. From a culture of eggs rayed at eighteen hours.

X-RAYS AND SOMATIC MUTATIONS

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IT is now evident that the experimental biologist may use to advantage X-rays and radium as tools for the study of his problems. The results already obtained by Stadler, by Hanson, and by Blakeslee and Buchholz on radium, by Stadler, Weinstein, Whiting and the various workers in our own laboratory on X-rays, have demonstrated the efficacy of this method of experimentation when applied to some of the major problems in biology. It is less than two years since Muller first announced his success in producing gene mutations and other genetic changes in the germ cells of Drosophila by means of X-rays, and yet within this short period a number of investigators have already obtained, by the same method, results of great importance. In the next few years we are certain to witness a very rapid development in X-ray and radium researches in biology.

My own investigations have centered on a study of the effects of X-rays on development, more particularly the effect of irradiation in the production of somatic mutations. In this short paper, I shall outline briefly some of the results already obtained on *Drosophila melanogaster*.

Somatic Mutations in Eye Color in Drosophila

I have already reported [7] some of the results obtained by raying eggs and larvae that were derived from flies heterozygous for certain of the sex-linked genes for eye-color. These results may be summarized briefly.

It was found that if the eggs and larvae derived from a cross between the normal, red-eyed female and a white-eyed mutant male were irradiated at different stages of development, a certain proportion of the flies, arising from the treated cultures, showed white ommatidial areas or patches on their eyes. The variant area reveals the recessive character and with but few exceptions, is made up of a definite number of white ommatidia. Two facts concerning these variant areas were soon discovered. First it was found that the size of the area was determined by the age at which the treatment was given, and not by the strength of the "dose" of X-rays employed. Second, it was observed that the variant areas were produced something like ten times more frequently in the females than in the males.

If eggs are treated during the first few hours of their development, the white area produced will be large (Figure 17 *A*, *D*). If the treatment is given during the mid-larval period, the white area will be composed of from ten to twenty ommatidia, and if given during the late-larval stages, it will be small—sometimes consisting of a single white ommatidium. If pupal stages are X-rayed no changes in eye color are produced. From this it was concluded that the change must be induced in a single cell of the eye rudiment, because otherwise it would be difficult to explain the variation in size of the different variant areas. Obviously, if the mutation occurs in one of the first two cells of the eye rudiment, approximately one-half of the eye would be white, because the white area would be amplified by cell-divisions of the affected cell. If the change is not induced until a later stage, then there would be fewer cell-descendants from the affected cell, and consequently a smaller white area will be produced.

That variant areas should appear about ten times more frequently in the femals than in the males was a result totally unexpected. An extensive series of experiments demonstrated that this ratio is practically constant. It can be explained best on the assumption

that all of the white areas found among males result from gene mutations, while only a few (about one-tenth) of those found in the females are due to gene mutations, the rest of the cases being due to the loss of the gene for red through a break in the X-chromosome. Evidently, the broken-off piece of the chromosome carrying the dominant gene is lost in the cytoplasm, or else enters the sister cell at a mitosis accompanying or following the breakage. This leaves the gene for white in the other X-chromosome free to determine the color of the resulting variant area.

We have also used other crosses, involving several of the allelomorphs of white, such as tinged, eosin and apricot. The results so far obtained have made it possible to make a better analysis of the behavior of a specific gene in development than by the use of the simple combinations of red to white. This is especially true in those cases in which the broken-off piece becomes attached to another chromosome (translocated).

For the first series of experiments, the eye was selected as the structure best suited for study, chiefly because it is made up of definite units, the ommatidia, and the data secured can be treated statistically. It was found possible to calculate the rate at which gene mutations take place after the application of an average dose of X-rays* to eggs and larvae. The calculations show that one gene for red ("W") in about every ten thousand (9891) mutates to white in the somatic cells of Drosophila. The frequency of breakage in the X-chromosome was also calculated, and was found, under the same conditions, to occur once in every 713 X-chromosomes that carry the dominant gene.

In a recent series of tests, in which over thirteen thousand control flies from the cross of red females by white males were examined for white ommatidia, eleven white areas were found. From these data we have calculated the frequency of breakage in the X-chromosome. There were ten females among the 6567 control females that had a total of 196 white ommatidia. The rate of breakage, calculated from these figures, is one break in every 57,680 X-chromosomes. Thus an average dose of X-rays of the magnitude above stated will cause the chromosome to break about eighty times more frequently than takes place spontaneously. There were 6472 males among the control flies. Only a single male showed white ommatidia (four). This number is too small to form a basis for the calculation of the rate of gene mutations occurring under natural conditions. The most interesting fact concerning these data is the ratio of ten females to one male showing white areas. This ratio is exactly the same as that found in the flies arising from treated cultures, although the frequency in the experimental material is many times greater.

Somatic Mutations in Body Characters

Mutations may also be produced in structures other than those belonging to the eye. Practically any recessive character may be made to appear as a variant area, provided the proper combination is used in making up the cultures to be treated. Several different combinations have been experimented with, but we shall refer to but three of them. In the first of these the mutant called "singed" was used. Singed is a sex-linked character, the gene for which is at point 21.0 on the chromosome map. It is an excellent body character to use because it affects not only the large bristles, but also the small hairs that are found on various parts of the fly. The hairs and bristles have the appearance of hairs singed by fire, and the presence of the character is very easily detected, even in a single bristle.

The cultures were made up by crossing yellow white females to eosin singed males. The yellow-white chromosome carries the gene dominant to singed.

*Eleven and one-half minutes exposure, at 50 K. V., 10 M. A., target distance 12 C. M.

while the other X-chromosome (from male) in addition to carrying the gene for singed will have gray, dominant to yellow, and eosin partially dominant to white. The F_1 females will be gray light eosin non-signed, while the F_1 males will be yellow white non-singed. From this it will be seen that if the X-rays will produce both gene mutations and breakages, three types of variant areas, showing recessive characters in question, may be expected. The females may show singed areas and yellow areas on the body, and white areas on the eyes; the males may show singed areas only. As a matter of fact, all three types were found in the F_1 flies developing from treated eggs and larvae. The white areas on the light eosin-eyed females will not be considered. Table I gives the results of the experiment, and shows the number of singed and yellow areas found.

In all, eighteen separate cultures of eggs and larvae were X-rayed, but in the table these have been combined into eight. The first culture (82-a) was given the D-2 dose (five minutes), all the others the D-3 dose (seven and one-half minutes). The term age in the second column means the age in hours of the eggs and larvae in the culture at the time of raying. The treated cultures gave 1137 females and 949 males. Eighty-seven female and one male showed at least one signed area each, and eighteen of the females showed yellow areas. About one female in every thirteen had a singed area, and about one female in every sixty-three had a yellow area. Singed areas appeared, therefore, 4.85 times more frequently than yellow areas.

The controls include the flies developed from all of the untreated cultures that were used in this and in the experiment on non-disjunction (see next section). All such flies were carefully examined for variant areas. There were 12,219 females and 11,201 males in the controls. There were found thirty-four singed areas and seven yellow areas on these females. In a few instances a given female had both a singed and a yellow area. There was one singed area for every 359 control females, and one yellow area for every 1745 such females. Singed areas, therefore, occurred 4.88 times more frequently than yellow areas in the control females. The ratio of singed to yellow is practically identical in the experimental and control flies. Irradiation does not change the ratio between the two types of variant areas, but does greatly increase the frequency of their occurrence.

The single male, in the experimental series, showing a singed area deserves further comment. The singed area on this fly involved the following bristles: the posterior scutellar, the post-alars, the posterior supra-alar, and about twenty hairs adjacent to the last three bristles, all on the right side. According to our interpretation, this area was produced by a gene mutation in one of the embryonic cells. Since there were 949 males in the series, evidently such mutations occur at rare intervals, even under the influence of X-rays. There was also found another variant area in one of the males. The area involved five bristles on the left side of the thorax, namely, the presutural, the humerals, and the notopleurals. The bristles in question were short and spike-like. This new somatic mutation very closely resembles a new germinal mutation, found in this laboratory by Dr. Muller, and called "prickly."

The size of both the yellow and singed areas follows the same rule as was found to hold in the case of white areas on the eyes. Flies developing from treated eggs or very young larvae show comparatively large variant areas (Figure 17 *B*, *E*, *F*), whereas, the areas on flies arising from larvae treated during the mid-larval stages are small (Figure 17 *G*). It not infrequently happens that the modified area consists of a single bristle, or of one or two hairs.

The facts obtained from this experiment indicate that since singed areas

are found more frequently than yellow areas, the yellow white chromosome must break more frequently than the gray-eosin chromosome. However, the difference in the frequency of breakage in the two types of chromosome is probably not as great as the data seem to indicate, because of the fact that singed areas are much more easily detected than yellow ones. A single singed bristle can be detected, while a single yellow bristle may be overlooked, especially if it happens to be located on the abdomen. This difficulty may be in part overcome, by comparing the number of cases of yellow and singed areas found among the large bristles on the thorax, where the chance of overlooking a single variant area is reduced to the minimum. A tabulation of all such cases shows that thirty-nine singed and eighteen yellow areas were observed and recorded. From this it is probable that the yellow-white chromosome breaks at least twice as frequently as the gray-eosin chromosome.

Experiments have been conducted that involved the use of factors on non-sex-chromosomes, or autosomes. In one experiment wild type females were crossed to ebony males. The gene for ebony is located at point 70.7 on the map of the third chromosome. Ten cultures were treated at different ages, and from these 223 females and 189 males were obtained. Three females and two males showed distinct "ebony" areas.

Another third-chromosome character used is that of spineless. Flies with this character have the large bristles much reduced in size. Yellow-white females were crossed to spineless-bithorax males. The gene for spineless is at point 58.5 on the chromosome map. The results obtained are shown in Table II. The treated cultures yielded 880 flies, of which 493 were females and 387 were males. Sixteen females and seven males had at least one spineless area each (Figure 17, *C*). Since, by the nature of the cross, the males are yellow, only females will show yellow areas. There were fourteen such females. None of the 866 control flies had spineless areas, but one female had a yellow area.

Non-Disjunction and X-Rays

In connection with certain experiments, designed to test the effect of irradiation on the production of mosaics and gynandromorphs, it was found necessary to "age" the female germ cells before giving the treatment. Among other things, it was found that aging the eggs in the female, which was to be irradiated, distinctly increased the number of exceptional individuals (due to non-disjunction) which develop from the treated eggs. The question of non-disjunction in Drosophila has been extensively studied by Bridges, Safir, Mavor, and Anderson. Mavor [5] has demonstrated that X-rays applied to the female increase non-disjunction, and Anderson [1] has confirmed his results. Since we do not expect, at least for the present, to follow up the work on the effect of aging and X-rays on non-disjunction, it seems worth while to present the data already obtained.

In this work several different combinations of sex-linked characters were used, and in each of these evidence was found that aging the eggs before X-raying them did increase the amount of non-disjunction. We shall, however, give the data on one combination only, because a fairly complete series of tests was made on it. The cross used was of yellow white females mated to eosin singed males. The reguiar F_1 females from this cross are gray eosin non-singed and the males, yellow white non-singed. The exceptional females are yellow white like their mother, and the exceptional males are eosin singed like their father.

The eggs were "aged" in the female by keeping the virgins for varying lengths of time before giving the treatment and mating. The virgin females were collected within four hours after the culture bottles had been freed of all flies. They were kept in shell vials

containing food. Usually about one hundred virgin females were placed in each vial. This number is used in order somewhat to crowd the flies, otherwise they will deposit some of their mature eggs. Even when kept under these conditions, the virgin females will deposit eggs, especially after the fifth day.

In the experiment in question, five different lots of virgin females were aged one, three, five, seven, and nine days, respectively. At the end of each period the females from one vial were etherized and divided into two equal groups. One group was given the D-3 dose.* The other group was used as controls. Individual matings were then made of both the treated and untreated females with eosin singed males. The parent flies were removed from the culture bottles at the end of the sixth day after mating. The F_1 offspring were examined and counted on the second, fourth, and sixth days after the flies began emerging.

In the treated series there were 218 pairs of flies, of which 198 were fertile. These gave in the three counts 11,384 females, of which 36 or 0.32 per cent were exceptional females, and 10,698 males, of which 172 or 1.61 per cent were exceptional males. In the untreated or control series, 190 matings were made, of which 171 were fertile. These yielded 11,643 females, of which 9 or 0.08 per cent were exceptional females, and 11,102 males, of which 20 or 0.18 per cent were exceptional males. As Table III shows, the greatest percentages of exceptions, after raying, occur consistently in the eggs from females aged 7 days after treatment. Furthermore, in each of the three sets of counts there was found to occur a gradual increase in the percentage of exceptional individuals up to and including the seventh day. There is but one exception to this, and that was found in the third count on the fifth day. After the seventh day (9 day count) there is a decided drop in the treated series. This is due, in all probability, to the fact that after the seventh day there is a strong tendency for the virgin females to lay their mature eggs.

Conclusions

This work has demonstrated that one of the main biological effects of irradiation is the production of breaks in the chromosomes. The method employed offers a means for investigating the action of a specific gene in development. The results obtained show that somatic modifications, similar in every way to those produced by gene mutations, are also produced by breaks in the chromosomes of heterozygous individuals. Furthermore, evidence is presented which indicates clearly that both kinds of "somatic mutations" occur normally, but at only a fraction of the rate at which they can be induced by X-rays. The X-ray method has, therefore, a distinct advantage over the natural method, in that the geneticist can now produce gene mutations and breakages at will, and in comparatively large numbers. He is thus able to analyze more easily and rapidly many of his problems.

A clear case in point has come up in connection with the work outlined above. In attempting to analyze the results obtained from a long series of experiments on the problem of breakage, it was found impracticable to determine, by the use of the standard stocks, whether or not the entire chromosomes (rather than a piece) had been eliminated. The problem is now in a fair way of being solved, thanks to a suggestion of Dr. Muller that I use one of his stocks having a "duplication." The stock in question has attached to the extreme right hand end of one of the X-chromosomes, a small piece carrying the factor for gray body color, derived from the left hand end of another X-chromosome. The experiment is designed in a manner such that a variant area resulting from X-rays

*Seven and one-half minutes, at 50 K. V. peak, 10 M. A., target distance 12 C. M., 1 mm. aluminum filter.

TABLE I—Yellow-White Females X Eosin-Singed Males

Culture	Age	Dose	Flies	Females	Males	Singed areas	Yellow areas
82-a	1-10	D-2	318	165	153	8 females	1 female
82-d	4-12	D-3	273	147	126	8 "	0
81-a	6-18	"	92	46	46	5 " ;1 male	2 "
81-b	12-24	"	165	77	88	9 "	0
81-a	24-36	"	641	377	264	28 "	10 "
81-g	26-45	"	149	79	70	12 "	2 "
82-f	36-48	"	264	160	104	8 "	2 "
82-g	51-63	"	184	86	98	9 "	1 "
Totals			2086	1137	949	87 " ;1 male	18 females
Controls			23420	12219	11201	34 "	7 "

TABLE II—Yellow-White Females X Spineless-Bithorax Males

Culture	Age	Dose	Flies	Females	Males	Spineless areas	Yellow areas
83-b	0-10	D-2	165	83	82	3 females	1 female
83-a	0-12	D-3	74	40	34	2 "	3 "
83-e	12-24	"	435	249	186	10 ",4 males	7 "
83-k	23-30	"	139	84	55	7 ",3 "	2 "
83-h	27-33	"	67	37	30	1 "	1 "
Totals			880	493	387	16 females,7 males	14 females
Controls			806	421	385	0	1 female

TABLE III—Shows the Effect of Aging the Eggs on the Production of Non-Disjunction by X-Rays

	First count- 2nd day		Second count- 4th day		Third count- 6th day	
Aged	Flies	% of non-disj.	Flies	% of non-disj.	Flies	% of non-disj.
1 day	2204	0.59±0.11	912	0.55 ±0.17	1301	0.38 ± 0.11
3 days	609	0.82 ± 0.25	1742	1.44 ± 0.19	1808	0.55 ± 0.11
5 days	1502	1.00 ±0.17	1759	1.59 ± 0.20	783	0.51 ± 0.17
7 days	2297	1.52 ± 0.17	1124	2.31 ± 0.31	790	1.01 ± 0,24
9 days	1695	1.12 ± 0.17	1872	0.48 0.12	1681	0.36 ± 0.10
		Control Series				
1 day	3041	0.10 ± 0.04	977	0.10 ±0.07	1547	0.13 ± 0.06
3 days	1755	0.28 ± 0.08	1833	0.11 ± 0.05	1598	0.00
5 days	2093	0.14 ± 0.05	1060	0.09 ± 0.06	684	0.14 ± 0.09
7 days	2527	0.11 ± 0.04	1198	0.17 ± 0.08	938	0.21 ± 0.10
9 days	1464	0.14 ± 0.07	1256	0.16 ± 0.08	777	0.00

will show whether a part or the entire chromosome is eliminated. The experiment is still in progress, but the results already obtained show that in each of the cases observed (eighteen) only a piece of the chromosome has been eliminated.

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Books Received

BOOKS are acknowledged in this column as received, and such acknowledgment must be regarded as sufficient return for the courtesy of the sender. As far as space permits, books that contain material of special interest to the readers of the JOURNAL *will be reviewed in later numbers.*

The Mothers, A Study of the Origins of Sentiments and Institutions: By Robert Briffault. In three volumes. Pp. 781, 789, and 841. 30 Chapters. Price (Sold only in set of three volumes) $20.00. Macmillan Co., New York, 1929.

Three volumes of Social Anthropology—enormously documented—with a bibliography of 196 pages. The author raises doubts as to the value of the rest of his work by undertaking to prove the thesis that heredity plays no part in social development, for the simple reason that mental and moral characters are solely produced by the social environment!

Six Talks on Heredity, A Handbook to the Cradle. By Mary Adams, M. Sc. Pp. 104. Six chapters. 20 Illustrations. Cambridge, W. Heffer & Sons, Ltd., 1929.

Scientific Method—Its Function in Research and in Education. By Dr. Truman Lee Kelley. Contains five lectures delivered by Dr. Kelley at Ohio State University. Pp. 190. Price $2.50. Columbus, Ohio, State University Press, 1929.

This book can be commended as an antidote for Briffault cited above. Includes an excellent discussion of the value and methods of education and of eugenics.

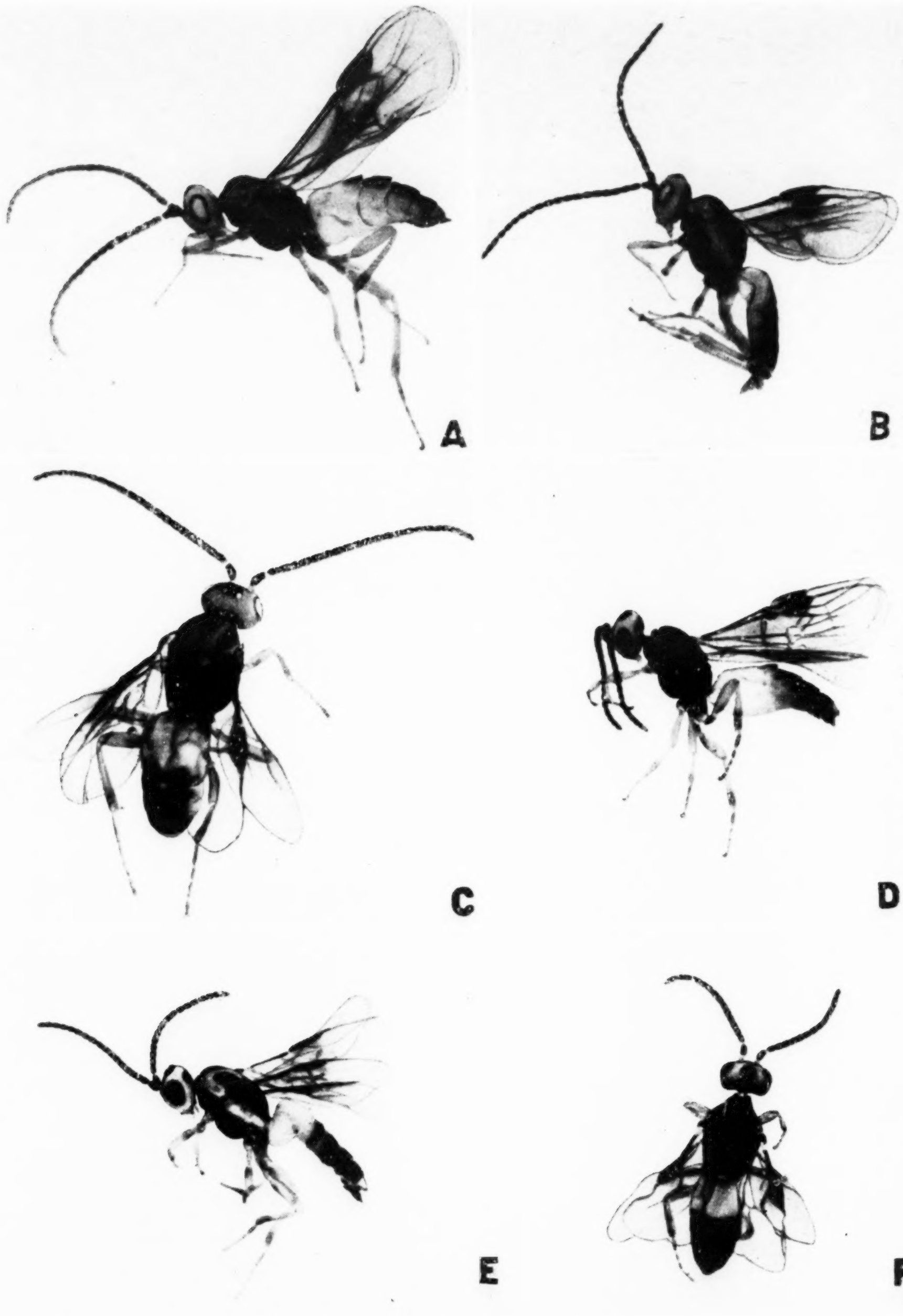
Die Deutschen und Ihre Nachbarvolker. ..By Prof. Dr. Walther Kruse. Pp. 640. 14 chapters. 17 illustrations and 5 tables. Leipsiz, George Thieme Verlag, 1929.

The Royal New Zealand Society for the Health of Women and Children. Annual Reports of Central Council and Dunedin Branch. Pp. 103. Dunedin, N. Z., Stone, Son & Co., Ltd., 1928.

New Zealand seems to take its children seriously. This organization must play an important part in the low infant death rate which characterizes this part of the "Antipodes".

Les Chromosomes, Artisans de l'Heredite et du Sexe. By Jean Rostand. Pp. 280. 22 Chapters. Paris, Librairie Hachette, 1928.

Textbook of Evolution and Genetics. By Arthur Ward Lindsay, Professor of Zoology in Denison University. Pp. 459. 25 Chapters. New York, The Macmillan Co., 1929.



MINIATURE—AN X-RAY MUTATION

Figure 18

A, ivory; *B* and *C*, ivory reduced; *D*, miniature; *E* and *F*, reduced miniature. Note the shortened antennae and smaller size of the wasps with the factor miniature. About fifteen times enlarged. Miniature arose as a mutation found in six of the sons of a male irrediated forty minutes. It is linked with ivory and is semi-lethal.

X-RAYS AND PARASITIC WASPS

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VISIBLE recessive mutations are much more readily detected in sex-linked factors than in non-sex-linked. A mutation occurring in the X-chromosome of Drosophila must come to light in the sons of any female carrying this mutation as a recessive regardless of the type of male to which she is mated. On the other hand autosomal genes may be by chance carried for several generations before the right combination is made to reveal them. The presence of sex-linked lethals is evidenced at once by decrease in number of males relative to females. Autosomal lethals may never be detected unless they happen to be linked with some trait for which counts are being made.

A species in which males are produced by haploid pathenogenesis, as in the case of bees, wasps, ants, white flies, and thrips, should show in the males recessive visible mutations occurring in any one of the chromosomes. Lethals, however, would not be as easily detected in this "sex-linkoid" inheritance as in the case of sex-linkage because there is not normally a constant sex ratio to be used as a standard with which to compare a decrease in males. In sex-linkoid inheritance sex ratio depends on number of eggs fertilized, which is in turn dependent on many and variable factors. Death of 50 per cent of the males, as would be expected in case of one lethal, is therefore difficult to detect. However, if two or three lethals be present and, especially if lethals be balanced in homologous chromosomes, there may be a noticeable reduction in number of offspring from virgin females or an extremely low male ratio from mated females.

Virgin females of the parasitic wasp, *Habrobracon juglandis* (Ashmead), produce haploid males, and most males from mated females are also haploid. Either mated or virgin females are placed in shell vials (vial a) with caterpillars of the Mediterranean flour moth supplied as food. After larvae appear(four or five days) the mothers are transferred to new vials (vial b) and this is repeated as many times as feasible or throughout the life of the wasp, frequently lasting for a month or more (through vials f, g, etc.). Productivity is very variable, in general being somewhat less during the first few days (vial a) than later (vials b, c, d) and subsequently decreasing (vials e, f, etc.).

Materials and Methods of Treatment*

Results of treatment of adult wasps only are reported in this paper. Investigations by treatment of immature stages are now in progress.

Virgin females are treated and immediately set without mating, or mated to untreated males before setting. Mated females are likewise treated, in which case sperm and eggs are simultaneously subjected to the rays. Males

*Experiments I, II, and III were made from February to June, 1927, under a grant from the Committee for Investigation of Problems of Sex of the National Research Council. The breeding work was carried out at the Bussey Institution and the exposures were made at the Massachusetts Institute of Technology. My grateful acknowledgments are due to Dr. J. T. Norton for use of apparatus and for many helpful suggestions and to Dr. Theodore Shedlovsky for arrangement of apparatus and for making the exposures. Experiments IV and V were made from March to June, 1928, with apparatus provided respectively by the Pittsburgh Skin and Cancer Clinic and by the Acme International X-Ray Company of Chicago. The breeding work was done at the Zoological Laboratory of the University of Pittsburgh. Technical help was furnished by a research grant from the American Association for the Advancement of Science. Experiment VI, both exposures and breeding work, was done in July, 1928, at the Marine Biological Laboratory, Woods Hole, Massachusetts.

are treated and then mated to untreated females which are subsequently set in vials.

The wasps are placed in gelatine capsules at approximately fifteen centimeters beneath the target of a Coolidge tube. Results of six experiments are reported in this paper. Conditions of current, voltage, etc., varied according to facilities available (See Table I). Target used in Experiments II and III was of molybdenum, in other cases of tungsten. A thin shield of aluminum served to cut out the softer rays. In any one experiment treatment of all wasps was begun at the same time. Current was turned off momentarily at intervals to remove some of the capsules. Time of exposure denotes the total time during which the wasps were actually being subjected to the rays.

General Results of Experiments

At the suggestion of Dr. H. J. Muller tests were made with dosages simliar to those that he had found effective in producing mutations and in inducing sterility in Drosophila. (Experiment I, Table I). Virgin females, mated females, and males were treated. No effect was produced on average number of offspring from first vials either in the case of virgin or of mated females. Ratio of female offspring was likewise not significantly affected.

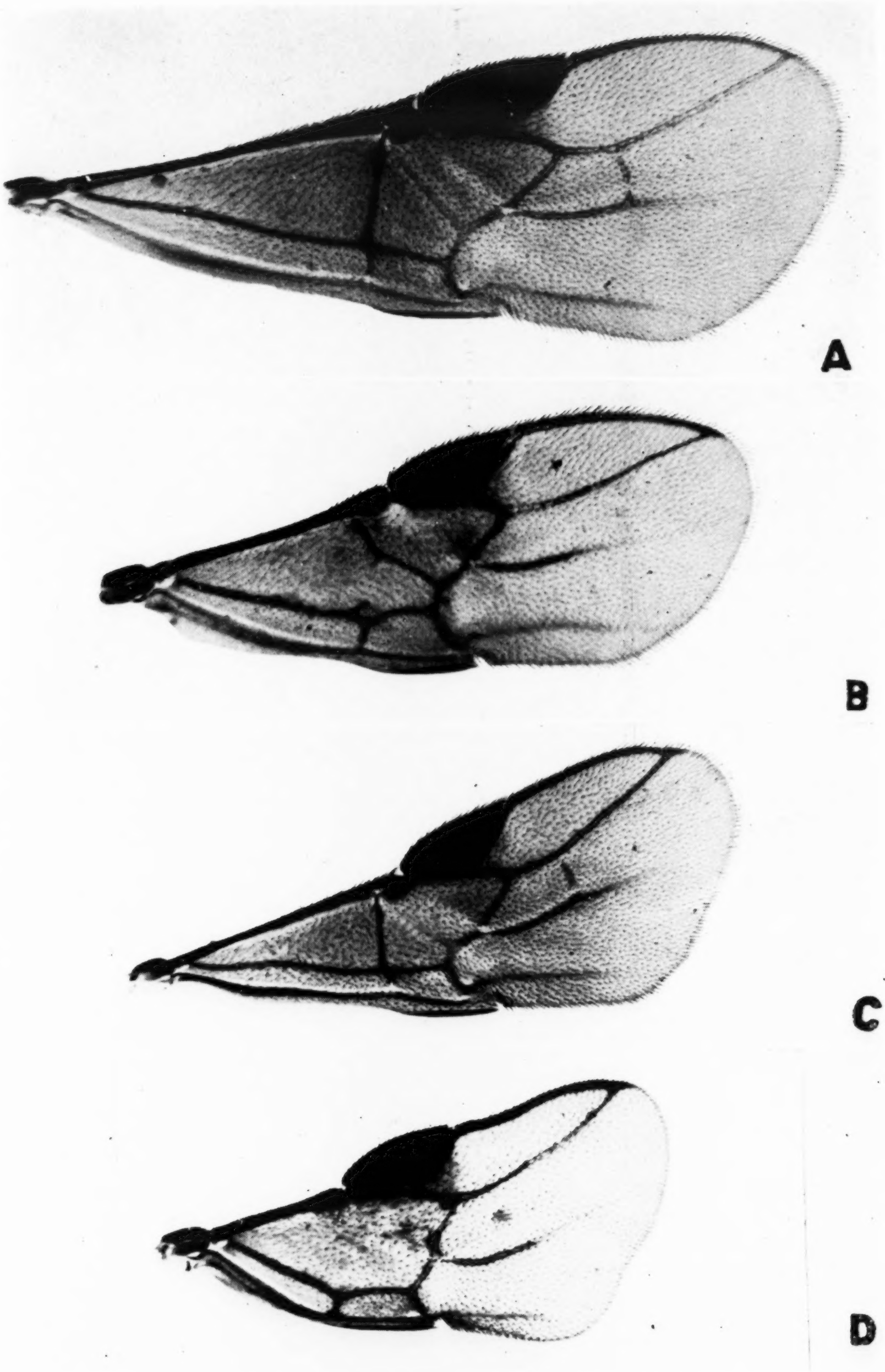
Treated males showed no influence of treatment. Average fertility as measured by female ratio among progeny was not significantly different in males exposed different lengths of time. The eleven exposed sixty minutes actually produced a higher proportion of females than the seven exposed twelve minutes. Twenty-five treated females and fifty-three treated males were tested in this experiment. No visible mutations appeared in the 517 male offspring of treated females or among the progeny of forty-five daughters of treated males and females that were tested.

This experiment was abandoned without completing the counts, as it was considered that the dosage had been too weak to give any results. Further experiments were planned to give a considerable range of dosage, in general much stronger than that which Dr. Muller had found effective with Drosophila but which had apparently proved entirely ineffective with Habrobracon.

In Experiment II (Table II) two classes of females were treated. Caterpillar-fed females had been with caterpillars for a few days previous to treatment and were hence in an actively egg-laying condition provided with mature and rapidly maturing eggs. Honey-fed females had been kept for some time on a diet of honey-water only and hence were without mature eggs. No consistent difference in duration of life appears to be associated with length of treatment. Among those exposed thirty minutes, seven of the caterpillar-fed showed much less tendency to become sterile than the other eight. These produced an average number of 123 offspring. All other females treated became sterile four or five days after treatment, although living a month or more. Among these there is, as the table shows, a consistent difference in fertility associated with the presence of nearly mature eggs, the caterpillar-fed being more fertile than the honey-fed.

This experiment, therefore, demonstrates that thirty minutes is a critical point for inducing sterility, but that this sterilizing effect is produced more readily on the less mature eggs. Even after ninety minutes' exposure there are a few fertile eggs produced by the females that have them ready to lay. No shortening of life by X-rays was produced.

In Experiment III the female wasps were freshly emerged from cocoons and were fed neither honey nor caterpillars prior to treatment. Results are summarized in Table III. From this table it may be seen that little if any



RIGHT PRIMARY WINGS OF MALES

Figure 19

A, normal; *B*, reduced; *C*, miniature; *D*, reduced miniature. Note reduction in size due both to miniature and to reduced. Note also reduction in venation due to reduced and modification in wing outline due to miniature. Enlarged about forty times.

lethal effect is produced on the treated females themselves, except possibly in the case of those treated two hours or more. Gradual reduction in duration of fertility and in number of progeny is seen with increasing dosage.

In Experiment IV (Table V) caterpillar-fed, mated females were used. Differences in treatment are not sufficient to affect average duration of life nor average number of progeny to any great extent. Complete sterility appeared after first five days after treatment except that among those treated forty minutes, three laid eggs after twelve days which developed into three adult males and two lethal larvae.

In Experiment V twenty-one young females that had been with males for some time were rayed for 120 minutes and immediately after treatment set with caterpillars. Untreated males were placed in the vials with nine of these females. Only two females appeared among the sixty-four offspring of the twelve females that were not with males after treatment, and only one female appeared among the thirty-eight offspring of the nine females that were with males both before and after treatment. Progeny were produced in the first vials (average 4.85+ per female) after which the females became sterile.

Males of Experiment III seemed to show no somatic effects of treatment, those that were treated 210 minutes being as active and mating as readily as those subjected to lower exposures. They were, however, completely sterilized after thirty minutes. Forty-two males treated thirty minutes or more produced no females among the 2,973 male progeny of their 108 mates.

Table IV summarizes results for males treated five, ten, and twenty minutes. Number of females produced among total progeny of their mates is taken as index of male fertility. Eighteen males were mated immediately after treatment, and on the fourth, seventh, and thirteenth days subsequently, being kept at room temperature. Six males were set in a cool place for fourteen days, when they were removed to room temperature and mated for four successive days. Observing percentages of daughters we note an almost consistent decrease of fertility with increasing treatment. We also note a consistent rise in fertility with days subsequent to treatment followed by a decrease with advancing age. The males kept at low temperature for fourteen days are more fertile than those mated immediately after treatment. They must also be regarded as physiologically young at their first matings. As in the case of the others, their fertility shows a tendency to increase as they begin life at room temperature and subsequently to decrease with advancing age.

In Experiment IV, only eight males were tested, but each of these was mated to a virgin female on each of six successive days after treatment, being kept meanwhile in the incubator at 26° C. Two males treated for sixty minutes and two for seventy minutes produced no daughters among the 1732 offspring of their twenty-four mates. Two that were treated for forty minutes produced thirty-four daughters among 596 offspring of twelve mates (5.7 per cent female ratio), while the two that were treated fifty minutes produced ten daughters among 731 offspring of twelve mates (1.37 per cent female ratio). Fertility is then decreased between forty and fifty minutes of treatment, while sterility is reached after sixty minutes of treatment.

Female ratios in fraternities summarized according to matings of these four males on the six successive days are 2.5+, 6.3, 1.7—, 5.5—, 1.3—, and 1.1+. There is a rise in fertility from the first to the second day after treatment and a decrease with advancing age as previously noted. The decrease in female ratio on the third day is consistent for each of the four males,

but may nevertheless be a chance fluctuation.

In Experiment VI a large number of females that had been with males was treated for different lengths of time. Altogether aberrant results were obtained indicating that a much weaker dosage was given than intended. Other investigators using the same apparatus at this time reported similar results, confirming the idea that dosage was in reality far below what the dials indicated (Table I, Experiment VI).

The only result of any interest from this experiment is in ratios of females which decrease markedly with increasing exposure. Fraternities were totaled in three groups (Table VI) according to length of exposure. Since there was a chance of error, however, due to the possibility that certain females were not mated and for this reason might be producing only males, it was thought worth while to exclude male fraternities from the totals, and to determine female ratios for the bisexual fraternities only. Results are in this case also according to expectation if some of the sperm in seminal receptacles of treated females were killed or rendered incapable of effecting fertilization.

In Experiment II among the offspring of mated females treated thirty minutes tests were made of ten daughters. Two gave no progeny, and two but one each, three produced a great excess of females (16 males to 101 females) while two showed nothing unusual (51 males to 62 females). The tenth gave rise to the mutation "small head" discussed below.

In Experiment III fifty-four daughters of treated females and seventy-seven daughters of treated males were tested. In several cases proportions of males were very low, suggesting that lethal mutations were present, but numbers were usually too small to be conclusive. Eight fraternities, however, having fair numbers totaled thirty-two males and 336 females, or over ten times as many females as males.

In Experiment IV fifty-three daughters of treated males and females were given a breeding test. Ten had an abnormally high female ratio indicating recessive lethals. One produced but five offspring (1 male, 4 females) although it lived about twenty days. One laid many eggs but only seven matured, all males. Another which laid many eggs produced one lethal larva and one mature normal female. One gave rise to the mutant, "miniature" discussed below. The remaining thirty-nine gave results which were apparently normal but some of these were inadequately tested.

One of the fraternities with an excess of females contained three males and forty-nine females. The mother was passed through six vials indicating that she lived twenty-four days or more. Besides the extreme sex-ratio, her fecundity is low. Seven of her daughters were tested. Three of these gave normal numbers of offspring. Two (giving four and five males respectively although they lived about fifteen days) probably were virgin and lethal bearing. The other two gave twelve males and eighty females indicating that they contained lethals but had paired with their normal brothers and were thus able to produce females in fair numbers.

In Experiment II one of the daughters of mated females treated thirty minutes produced in addition to twenty-eight normal females and nineteen normal males, twelve males with unusually small heads. Tests of eleven of these normal females showed four producing normals only (52 males and 35 females) while seven produced small head sons (37 normal, 12 small head). Four of these gave small head daughters also (15 normal, 11 small head). The trait was especially well marked in the females, although somewhat intergrading in the males. Only those with very small heads were classified as small head. This method of classification accounts in all probability for the excess of normals. Un-

LETHAL MINIATURE PUPAE

Figure 20

A variable proportion of miniature die before maturity but some develop into pupae with distinguishable eye color. Enlarged about fifteen times.

fortunately on account of temporary interruption of the work the small head stock was lost.

One of the females treated for seventy minutes in Experiment IV, from pure orange-eyed stock, produced six sons, five of expected orange eye color and a mutant with eyes indistinguishable from ivory. Wild type black, *O*, light ocelli with black eye, o^1, orange, *o*, and ivory, o^i, form a quadruple allelomorphic series. Since ivory arose originally from orange stock, it was supposed that this mutant male from the treated female might be a second mutation from orange to ivory. As a test he was accordingly mated with females from ivory stock. He produced, not ivory daughters as expected, but orange, and when he was subsequently mated with these daughters produced in addition to orange, daughters with eyes that were ivory in appearance. These results may be expected if the original mutant was not ivory but a second recessive, "cream", resembling ivory in appearance but caused by a change in a different locus. When cream males were crossed with black females the black daughters resulting produced black, orange, and cream sons. This fact is consistent with the hypothesis of an independent locus but ratios are very aberrant, differing widely in different cases and indicating further complications. Moreover there are at least two different types of orange, light and dark, arising from crosses of cream with black. Further analysis is being made.

One of the males treated for forty

minutes in Experiment IV, mated on each of six successive days, produced two females among the seventy progeny of his last mate. One of these females produced, in addition to nine normal, six rather small males with wings of somewhat aberrant shape; and antennae somewhat shortened. This mutation, miniature, has proved to be linked with the locus for orange eye color, giving about ten per cent cross-overs. The trait is semilethal, only about half as many miniature as normal maturing among the progeny of heterozygous virgin females. Of those that die before maturity a small and variable percentage reach a stage of pupal development late enough so that it is possible to determine eye color. Ten per cent crossovers may therefore be counted not only in the normal adults and in miniature adults but in the lethal pupae as well.

Figures 18 to 20 show F_2 males bred parthenogenetically from virgin females of the constitution $\frac{o^i \ M}{O \ m} \ \frac{r,}{R}$ derived from cross of female with ivory eyes, o^i, reduced wings, r, and large body, M, by male with black eyes, O, long wings, R, and the X-ray semi-lethal mutation miniature body, m.

Summary and General Conclusions

There appears to be little if any effect of X-raying on the duration of life or general vitality of Habrobracon.

Females are sterlized but produce a few offspring immediately after treatment, probably because mature eggs are relatively resistant. If the females are fed on caterpillars previous to treatment so that they are actively laying eggs they are more likely to retain fertility than if they have recently emerged from cocoons or have been kept alive for a period by being fed with honey. After strong dosages the caterpillar-fed females produce a few offspring immediately after treatment and then become sterile. Young or honey-fed females treated with weak dosages either retain fertility or produce a very few offspring immediately after treatment and then become sterile. After strong dosages they produce no progeny at all.

Treatment of mated females apparently decreases ratio of females among their progeny, probably by injury to sperm in seminal receptacles.

Fertility of treated males is measured by female ratios among the progeny of their untreated mates. Males are sterilized by strong dosages and their fertility is reduced by weak dosages. Fertility of treated males rises from first to fourth day after treatment and drops as males become older. If treated males are kept in a cool place for a few days after treatment and thereafter mated and kept at room temperature, fertility is greater than if they are tested immediately after treatment; but in this case also fertility first rises and then falls during life at room temperature.

As regards production of mutations or other hereditary changes treated females have shown very little of interest in their male offspring. Only one visible mutation was found, "cream". This apparent resistance may be due to one or more of the following reasons:

(1) The germ plasm may be relatively stable.

(2) Somatic variability may obscure genetic changes. Body color varies from yellow to black on account of temperature. Many non-hereditary freak forms occur. These conditions may cause certain mutants to be overlooked.

(3) There may be required a considerable germinal change in order to affect a certain character, as for example eye-pigment.

(4) Potentially visible mutations may be produced but simultaneous lethal mutations may occur in the same or in any other chromosome. Sterility of treated females may be in part explained by these lethals. As further

evidence of lethals may be cited the presence of lethal larvae especially among the later offspring of females that recover a certain amount of fertility.

Female offspring of treated males, as also female offspring of treated mated females have shown evidence of carrying lethals as evidenced by their low fecundity or the low male ratio among their offspring. Of one hundred and forty-one tested offspring of treated wasps, two gave visible mutations, "small-head" and "miniature" while at least 28 showed conditions indicating lethals. It is very likely that some of the others likewise carried lethals but tests were inadequate in view of the normal variability of the sex ratio.

TABLE I—Conditions of Experiments

Experiment	Aluminum Shield (mm)	Kv.	Miliamperes	Exposure (minutes)
I	1/3	50	5	12. 24. 36. 48. 60.
II	"	40	25	30. 60. 90.
III	"	40	20	5. 10. 20. 30. 60. 105. 150. 210.
IV	½	45	8	40. 50. 60. 70.
V	1	50	8	120.
VI	"	40	10	30. 45. 60. 75. 90. 200. 300.

TABLE II—Experiment II, Treated Females

	Caterpillar-fed Females				*Honey-fed Females*		
Exposure (minutes)		30	60	90	30	60	90
Number treated	7	8	7	7	16	3	2
Av. Duration of Life (days)	36—	27	30—	40+	29+	31	30
Av. Duration of Fertility (days)	22—	6—	6—	6—	6—	0	0
Av. Number Progeny	123+	10+	7+	2+	5—	0	0

TABLE III—Experiment III, Treated Females

Exposure (minutes)	5	10	20	30	60	105	150	210
Number treated	7	10	12	8	3	4	12	7
Av. Duration of Life (days)	24—	36+	34+	39	39	22	19+	11—
Av. Duration of Fertility (days)	22—	13+	13+	6—	0	0	0	0
Av. Number Progeny	44	11+	22+	4—	0	0	0	0

TABLE IV—Experiment III, Treated Males

Exposure	No.	*Males mated immediately after Treatment* Days after Treatment 0 Total	0 % ♀	4 Total	4 % ♀	7 Total	7 % ♀	13 Total	13 % ♀	No.	*Males kept in cold fourteen days before mating* Days after Treatment 14 Total	14 % ♀	15 Total	15 % ♀	16 Total	16 % ♀	17 Total	17 % ♀
5	5	19[illegible]	22—	155	40	302	16+	269	17	3	115	38+	91	50+	135	51+	81	42—
10	5	229	15—	135	22+	169	14+	238	2+	2	76	28—	65	51—	37	30—	48	25
20	8	250	6	114	18—	113	8—			1	104	12—						

TABLE V—Experiment IV, Treated Females

Exposure (minutes)	40	50	60	70
Number treated	18*	23	17	17
Average Duration of Life (days)	24—	27—	32+	34—
Average Number Progeny	5.8+	3.6—	3	4

* Three recovered slight fertility after twelve days and produced three adult males and two lethal larvae.

TABLE VI—Experiment VI, Treated Females

Exposure (minutes)	Total Progeny	% ♀ ♀	Total in Bisexual Fraternities	% ♀ ♀
30 to 60	2506	21.2+	2227	24.0—
75 to 90	1739	15.1—	1554	16.9—
200 to 300	1045	9.1—	812	11.7—

VISIBLE MUTATIONS FOLLOWING RADIUM IRRADIATION IN DROSOPHILA MELANOGASTER

FRANK BLAIR HANSON and ELVENE WINKLEMAN
Washington University, St. Louis, Mo.

THE years 1866, 1900 and 1927 constitute a notable triumvirate of dates in the history of Genetics.

In the first of these years Mendel published his famous paper on inheritance in the edible pea; 1900 witnessed the dramatic rediscovery of Mendel's laws by Correns, de Vries and Tschermak; and in 1927 Muller announced that gene mutations could be produced in abundance in the fruit fly by means of X-rays.

The artificial transmutation of the gene is an old problem. Many investigators have tried their hands at it. But like the attempts at the transmutation of the baser metals into gold the genes, for the most part, remained stable. Yet in rare instances natural mutations ocurred without a discoverable cause.

If, as most geneticists believe, mutations are the "building blocks" of organic evolution the natural rate of mutation is too slow for an adequate analysis of the process. Mutability as practiced by nature is of the order of a slow motion picture film—incredibly slow as measured by the years of the investigator. How can nature's film be speeded up? All attempts failed to accomplish this in anything like an adequate manner until Muller applied X-rays to the fruit fly. In some of his experiments the rate of mutation among the offspring of irradiated parents was 15,000 per cent greater than the normal rate in his controls.

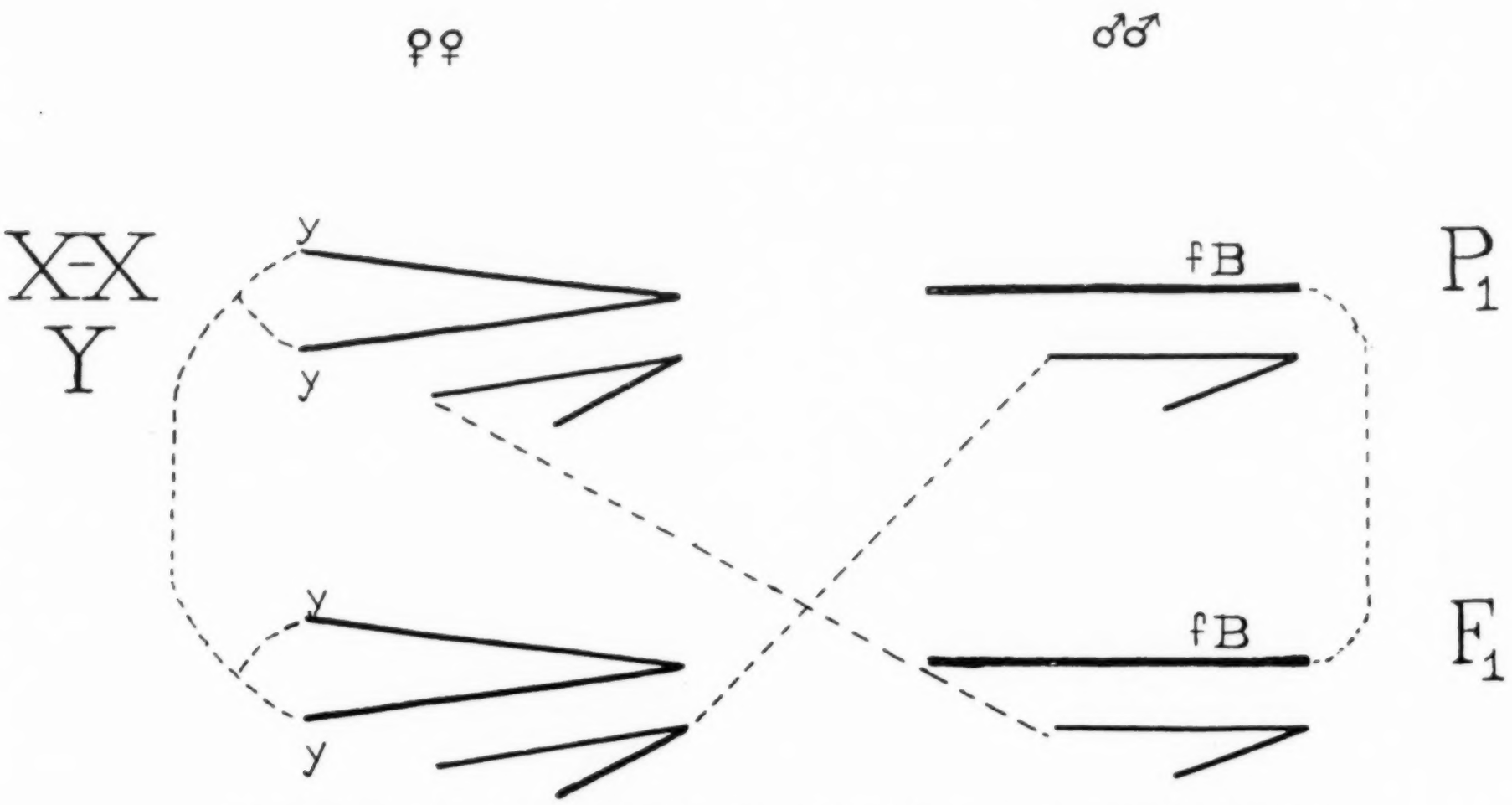
Radium Experiments

Radium is as effective as X-rays in causing mutations in Drosophila flies. Hanson and Heys[1] have shown that when flies were treated for six hours with 140 milligrams of radium the percentage of lethal mutations was 8.2 per cent. And, further, that when the alpha and beta rays were completely absorbed by a lead screen the gamma rays produced 2.8 per cent of lethals. Stadler (1928) found three mutations in radiated barley seedlings out of 1039 head progenies examined.

Visible as well as lethal mutations are produced in Drosophila by radium. This paper attempts nothing more than to put this fact on record. As will be seen from the tables given below many visible mutations have been found following radium irradiation. As is also the case with X-rays many of the mutants are somatic only and the abnormality perishes with its possessor; others, in addition to some visible mutation, are sterile and no further study is possible. But reference to Table II shows that out of a total of 204 visible departures from the normal 43 or 21.07 per cent were germinal variations; 80 were sterile and 81 failed to breed true.

Dosage and Breeding Methods

Three experiments were performed with slight variations in dosage. Forked-Bar males were exposed to the radium rays. During treatment they were confined in shell vials $2\frac{1}{2} \times 1$ inches. One inch of a banana-agar food was put into each treatment vial and the open ends were covered with two layers of gauze. The radium needles were attached with adhesive tape to the gauze over the open ends of the vials. In the first two experiments 150 milligrams of radium were used; in Experiment III, 140 milli-



SCHEME OF CROSSINGS IN "DOUBLE-X EXPERIMENT"

Figure 21

Y represents Y chromosome in double-X females; y represents mutant gene for yellow body located in the X-X, or double-X chromosome. The sons get their X-chromosomes from their fathers, and mutations appear at once, whether dominant or recessive, as the Y-chromosome does not carry normal allelomorphs. (After Muller.)

grams were used, one 10-mg. needle not being available at that time. The other two elements in the dosage, time and distance, are given in Table I.

Immediately following treatment these males were mated to virgin double-X yellow females. These females are peculiar in that the two sex chromosomes are attached at the right hand end and go together into the same gamete, which is equivalent to 100 per cent non-disjunction. The double-X yellow females also carry a male Y-chromosome. In such a cross the sons get their X-chromosome from their fathers and their Y-chromosome from their mothers, a reversal of the usual procedure in this species. There are several advantages in using a stock made up in this way. Practically all mutations occurring in the sex chromosome of the treated fathers show up in the first generation of sons, whether recessive or dominant, as they are not covered by normal allelomorphs in the Y-chromosome.

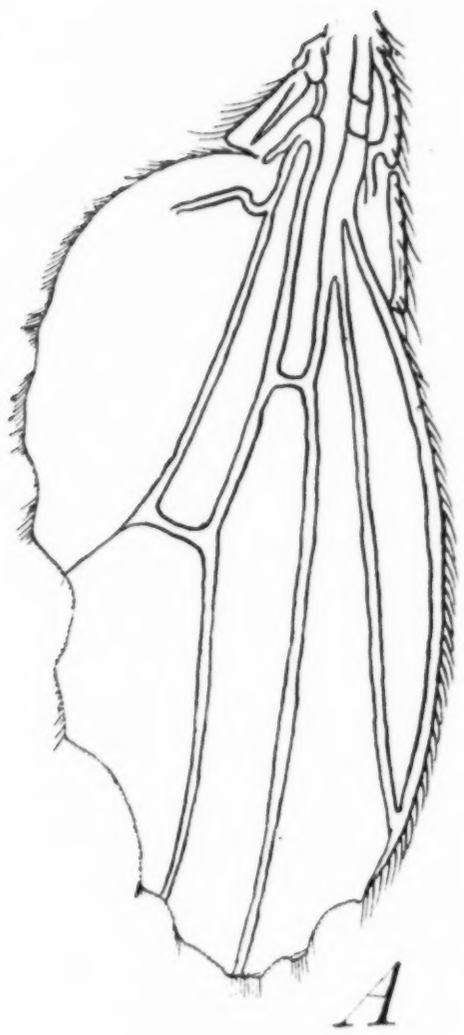
Figure 21 gives the scheme of crossings of treated males with double-X yellow females.

Experimental Results

Tables I-III give the results of the first three experiments in which the cross was made between forked-bar males and double-X yellow females. With the exception of slight differences in dosage the first three experiments were identical. Table IV gives the results of a search for visibles in offspring produced in an experiment designed primarily to test the production of lethals from radium, the work on visible mutations being merely a by-product.

There were three broods raised in each of the first three experiments. Brood I constitutes the offspring of the first seven days of egg-laying after mating. The parents were then transferred to new food bottles and Brood II came from the eggs laid the second seven days. Similarly with Brood III.

Variations in time of exposure and number of milligrams of radium are indicated in column 2 of Table I. The total number of bottles started in each brood of each experiment follows in the same table, together with the number of pairs of parents per bottle. This varied from four pairs in Experiment



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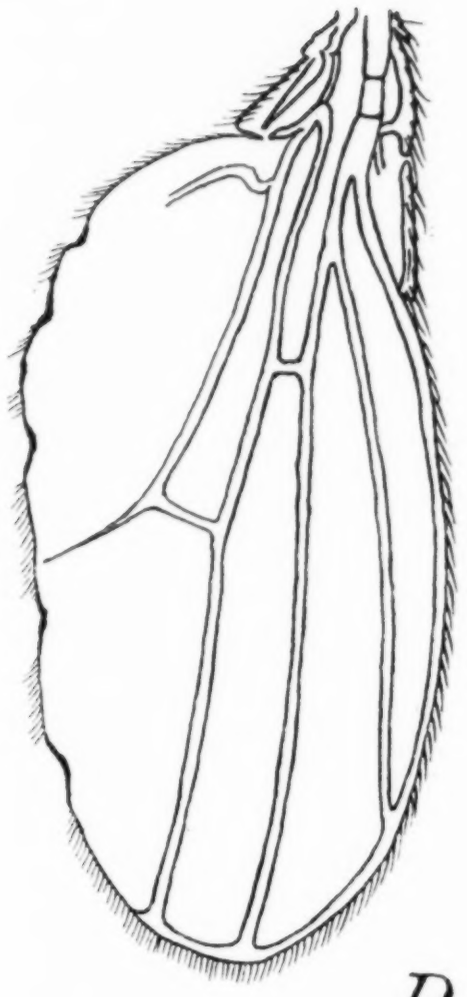

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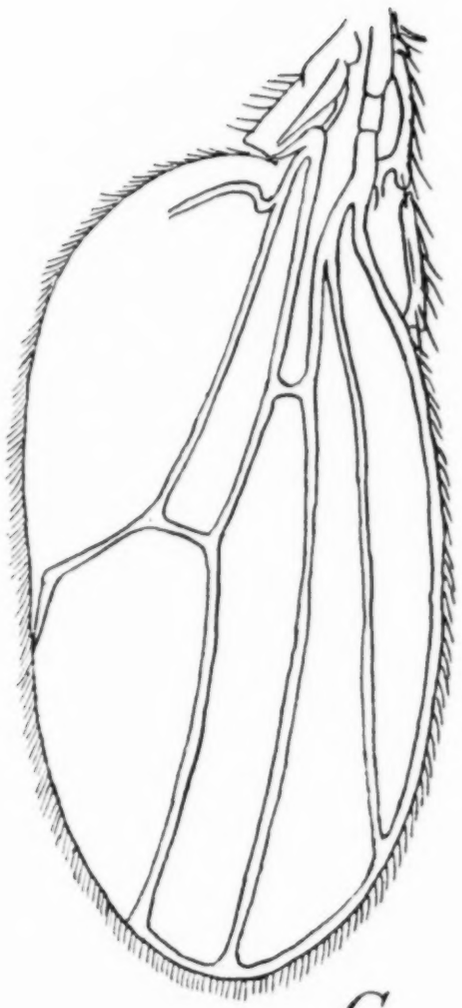

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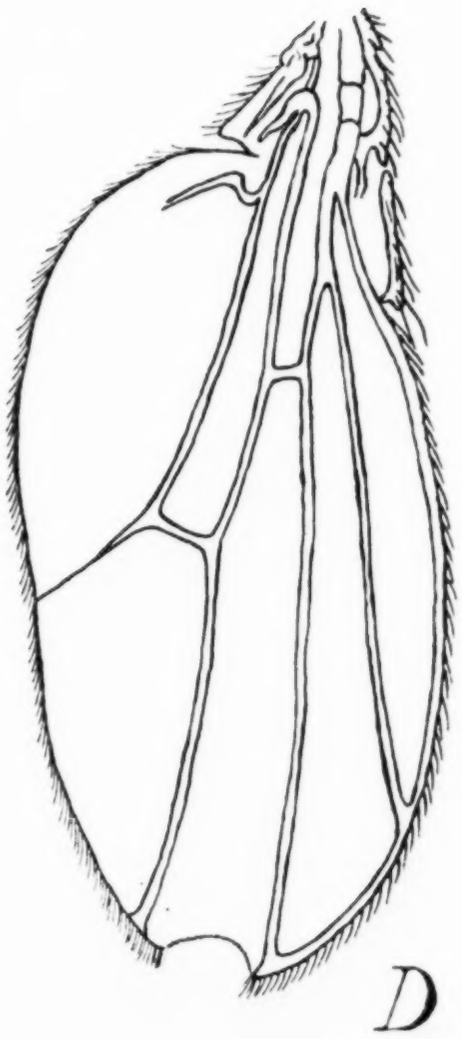

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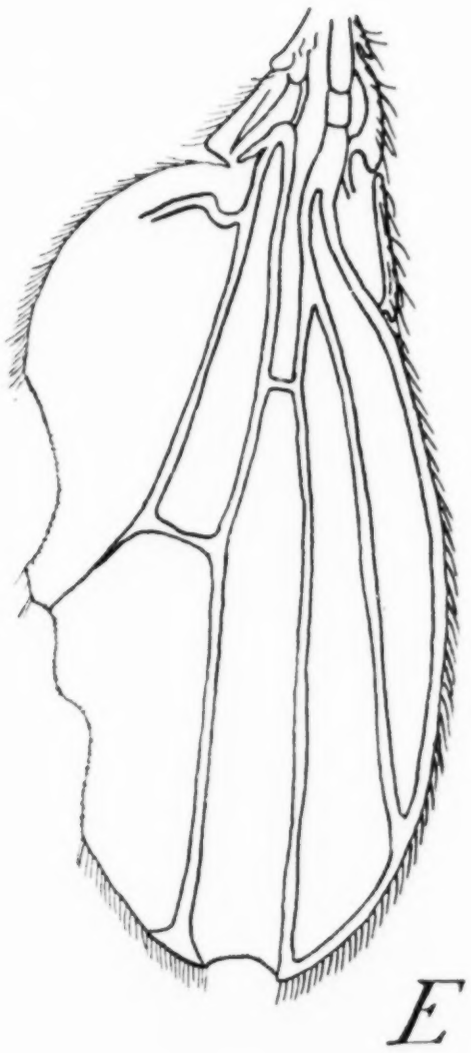


E

WING MUTATION

Figure 22

A, cut; *B*, edge; *C*, angle at the end of the fifth longitudinal vein; *D* and *E*, variations in notch.

MINUTE BRISTLES
Figure 23

A gene that reduces the forked bristles of this stock in the same way that *minute* reduces normal bristles.

I to eight and ten pairs in Experiments II and III respectively.

Since all sex-linked mutations, dominants as well as recessives, show up in the F_1 males of a cross between radiated fathers and double-X yellow mothers, mutations were looked for only in the male offspring. The total number of male offspring in the first three experiments was 14,480, among which were noted 204 cases of visible abnormalities. The distribution of these 204 abnormal males among the various broods is shown in Table I, together with the percentage of defectives to the number examined.

In all three experiments the per cent of mutations falls off markedly in the third brood. This is of interest in connection with unpublished results on the number of lethals occurring in successive broods. There it was found that after two broods, or 14 days of egg-laying, the per cent of lethals dropped sharply, almost to 0. It would seem that early germ cells are not so susceptible to irradiation as the mature sperm or those nearly so. Since most Drosophila workers will not catch all the visibles present and may vary from day to day in the keenness of their perception, these percents would not mean much by themselves, but taking into consideration the fact that all three experiments are consistent in this regard and the results are also in line with our experience with lethals where quantitative studies are very exact, the conclusion seems warranted that in the fruit fly the results of raying last not longer than two weeks. In two of the experiments reported here the mutation rate also falls off for the second brood. But this is not in accord with the results on lethals, where the rate in Brood II in a number of tests is always equal to that of Brood I, the sharp drop coming between Broods II and III.

Table II is an analysis of the 204 visible abnormalities found. In all broods 80 were proven sterile and discarded. Eighty-one were somatic mutations, for upon breeding to double-X yellow females they produced normal sons. But 43 were true germinal mutations and an analysis of their germinal constitution is under way.

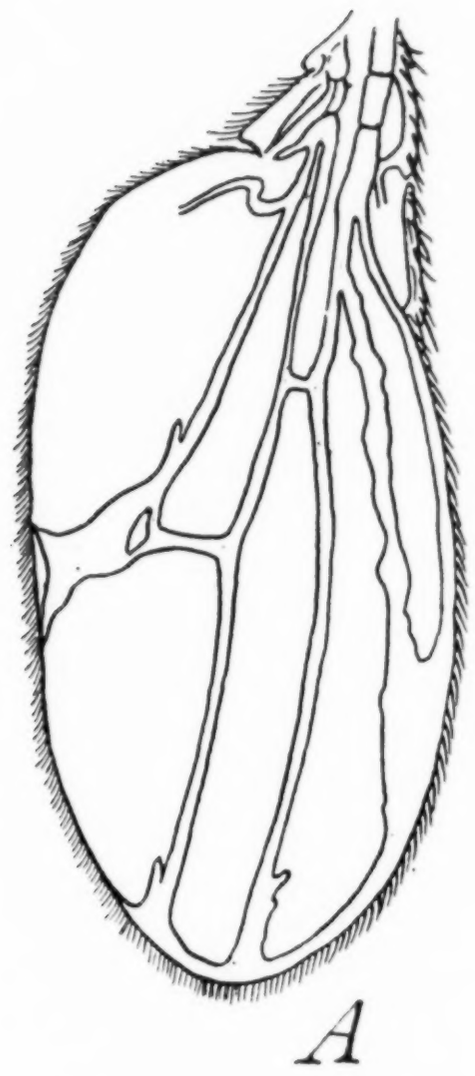
Table III lists the 43 germinal mutations found in the first three experiments. These are arranged to show the number of times each occurred and their distribution between the X-chromosome and the autosomes.

Mutations in the X-chromosome

All of the fifteen mutations listed as "X-Chromosome Mutants" in Table III bred true when mated to double-X yellow females, this being the proof that they are sex-linked. Analysis of these mutants is now under way to determine the position of each gene in the chromosome. The following are brief descriptions of the X-chromosome mutations:

Miniature wing: This is the same as the original miniature. In addition the wings are very dusky in appearance. (Figure 25 *C*).

Bar to round eye: This occurred five times and is unquestionably a return gene mutation. In addition the senior author has four more cases of return to round eye. All nine return mutations took place in the male when crossed to double-X females, hence cannot be explained as due to unequal crossing-over as is the case in the bar female.



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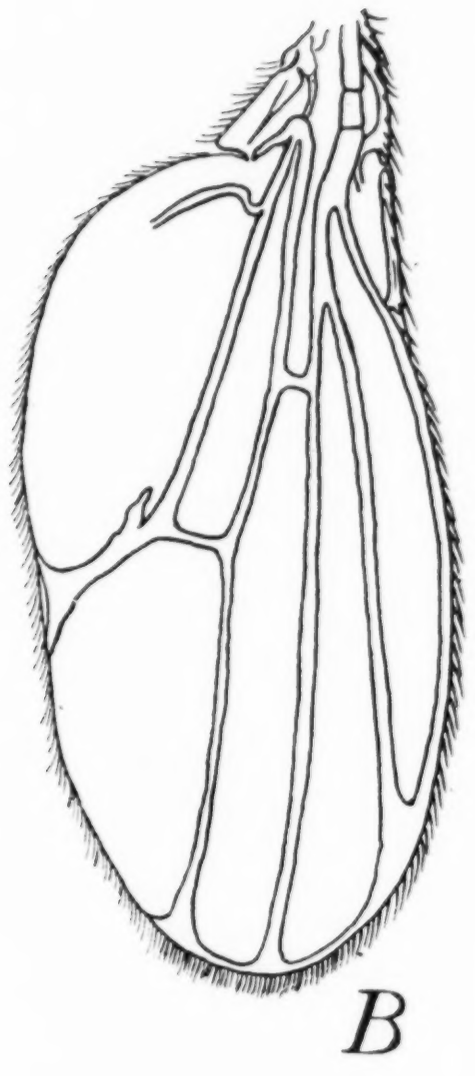

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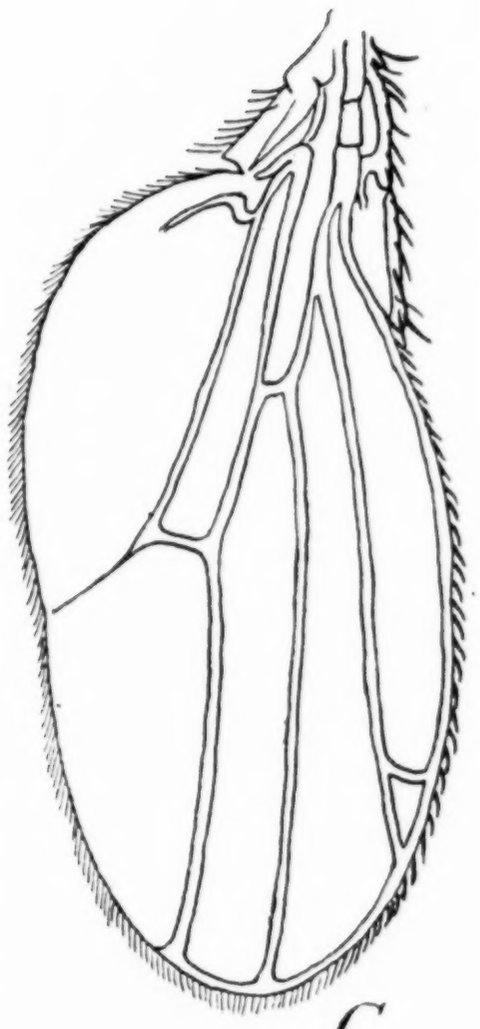

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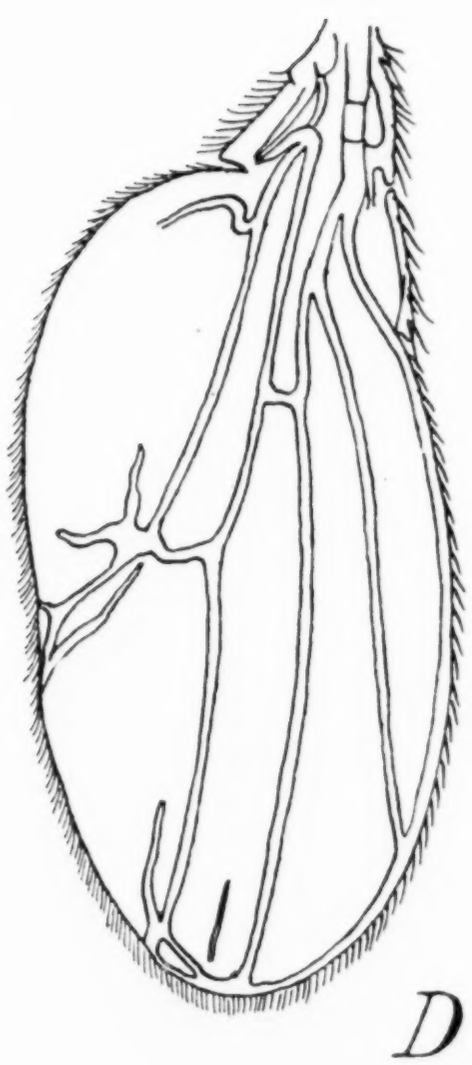

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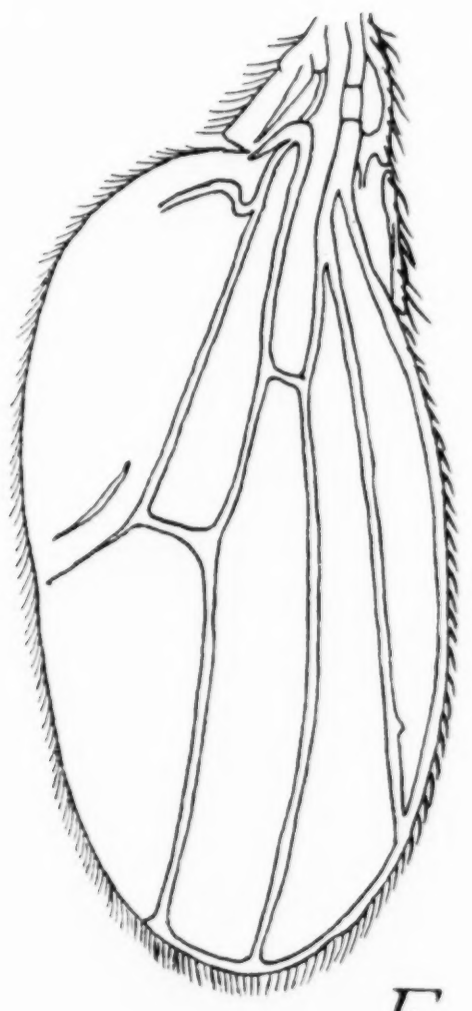
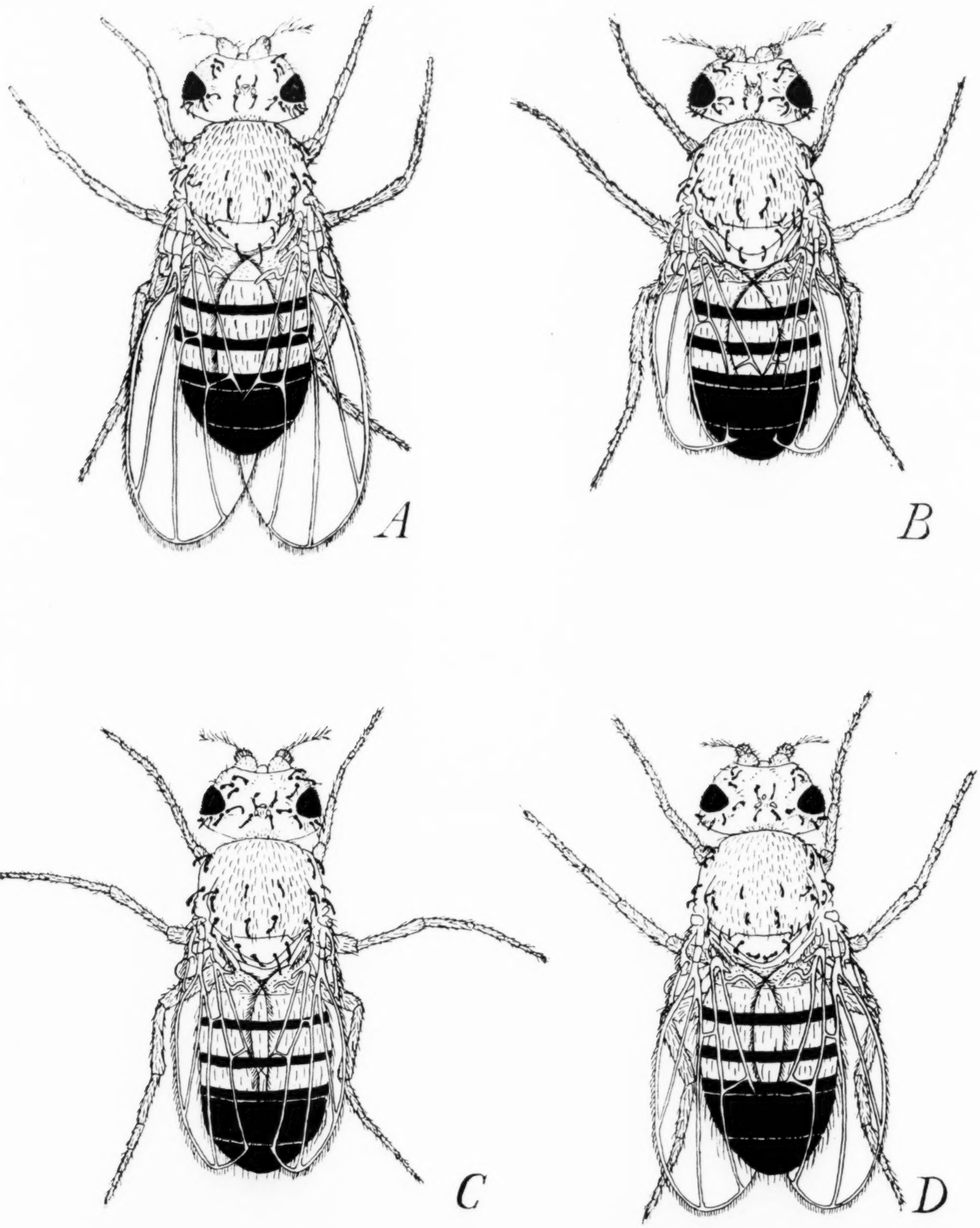


E

MUTATIONS IN WING VEINS

Figure 24

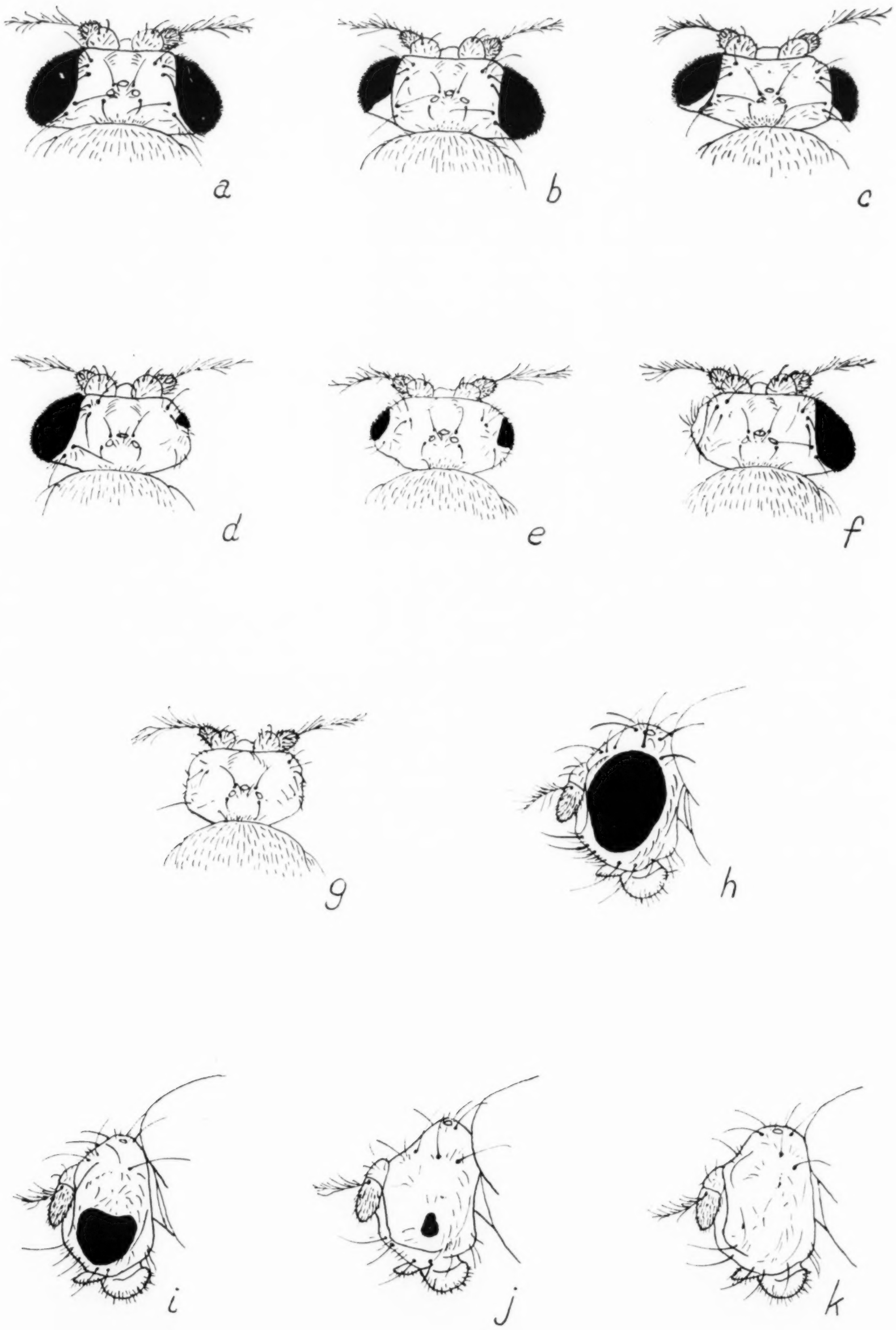
A. Delta large; *B*. Delta small; *C*, Branched; *D* and *E*, variations in plexus.



MUTATIONS IN SIZE OF WING

Figure 25

Wing mutations compared with normal wing. *A*, normal; *B*, rudimentary; *C*, miniature; and *D*, small.



EYELESS MUTATIONS COMPARED WITH NORMAL

Figure 26

a, normal eye, top view; *b*, *c*, *d*, *e*, *f*, and *g*, top views of different degrees of eyeless. *h*, normal eye, side view; *i*, *j*, *k*, side views of different degrees of eyeless.

Notch: This is similar to notch previously described. It is terminal and occurs in one or both wings.

Edge: Edge is a wing mutation in which there are thickenings along the inner margins of each wing. This gives the wing a slightly ruffled appearance. These flies have extremely poor viability. (Figure 22 *B*).

Cut: A recessive similar to the *cut* previously described. The marginal vein is cut at the tip of the wing and the inner margin is scalloped. Viability very poor. (Figure 22 *A*).

Small wing: The wings are about four-fifths of normal length. Poor viability. (Figure 25 *D*).

Rudimentary: A recessive similar to other rudimentaries. The fourth longitudinal vein in usually broken and sometimes the posterior cross-vein. Is somewhat variable both in shape and venation. (Figure 25 *D*).

Truncated: Name given when first found, but is probably identical with *rudimentary* described above.

White-Eye: This was found twice and is the same as the well-known mutant of that name.

Autosomal Mutants

All of the autosomal mutations are dominants. Only the F_1 generation was examined and since autosomal recessives do not come out until the F_2 generation these must have been dominants and not sex-linked. Following are brief descriptions of these dominants:

Delta: This is a wing mutation characterized by irregular thickenings of the longitudinal veins and delta-like expansions in the veins where they join the marginal veins. Large Delta is distinct from small Delta. Three less extreme forms were also found which overlap the normal. (Figure 24, *A* and *B*).

Branched: Shows an inconspicuous branching of the second longitudinal vein where it joins the marginal vein. (Figure 24, *C*).

Outstretched: Has the wings extended at right angles to the body.

Curly: This has the wings bent up and forward at the tip like the well-known *curly*.

Plexus: Plexus is extremely variable. Usually shows a network of veins at the junction of the fifth longitudinal and posterior cross veins. Other veins may show irregularities also. Several stocks show both *Plexus* and *Branched* and it is expected that these will prove to be caused by the same gene. (Figure 24, *D* and *E*).

Wrinkled. Has wings irregularly creased or folded.

Notch: An extreme form in which the wings are deeply cut.

Vicinal: The third and fourth longitudinal veins are much closer to each other than in the normal wing.

Blistered: May show in one or both wings. The vesicles or inflations are large and the venation is irregular.

Uneven Edge: Shows slight thickenings on the inner margin of each wing.

Crumpled: One or both wings are rumpled in appearance. The wing may be depressed or not fully expanded.

Minute Bristles: This mutation reduces the forked bristles in the stock used in the same way that *Minute* reduces normal bristles. Whether the same gene or not has not been determined yet. (Figure 23).

Angle: A rather constant venation irregularity. The fifth longitudinal vein makes an oblique angle just before it reaches the inner edge of the wing. (Figure 22, *C*).

While this work was under way the senior author was carrying on an experimental study of the production of lethal mutations by radium radiation. One lot of these flies whose parents had been treated with 150 mg. of radium, the rays being passed through a lead filter .005 inch thick, was turned over to the junior author to examine for visible mutations. Table IV gives the results of this examination.

Visible Mutations from the "Lethal" Experiment

Notch: An X-chromosome recessive which overlaps the normal type. Shows

considerable variation. Preliminary tests indicate that it is due to a gene near the middle of the X-chromosome. (Figure 22, *D* and *E*).

Outstretched: Extremely poor viability. Since lost. The wings were extended at right angles to the body.

Roughoid: The eye had a rough appearance due to facet irregularities. Lost due to poor viability.

Eyeless: Variable like the *eyeless* of the fourth chromosome. The range of variability is from a scarcely perceptible diminution in the size of one eye to completely eyeless on both sides. Both normal and bar eyes are reduced. (Figure 26).

Cream eye: This is an autosomal eye-color mutation. The color of the eye varies from a deep cream to a light peach color. It behaves as a dominant but is not lethal when homozygous.

Conclusion

This paper reports a total of 49 germinal mutations arising from males treated with radium and mated to double-X yellow females or from the ClB stock of an experiment on lethal mutations. They have, at the present writing, been separated into sex-linked and autosomal groups and for such of them as can be kept going an analysis of their position on the chromosome map will be made in a later paper.

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1. Hanson, Frank Blair, and Florence Heys. 1928. The Effects of radium in producing lethal mutations in Drosophila melanogaster. *Science*, 68:115-116.

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3. Stadler, L. J. 1928. Mutations in barley induced by X-rays and radium. *Science* 68:186-187.

TABLE I—A table giving the various details of a cross between radium radiated forked-bar males and double-X yellow females in the three experiments

		Hours treated	No. bottles	Prs. per bottle	♂♂ counted	Visible abnormalities	
				Experiment I			
Brood	I	6	43	4	358	16	.045
Brood	II		47		347	6	.017
Brood	III		49		1,216	10	.008
				Experiment II			
Brood	I	5	104	8	1,178	52	.044
Brood	II		100		1,978	16	.008
Brood	III		100		2,925	7	.002
				Experiment III			
Brood	I	5¼	100	10	1,989	41	.021
Brood	II		99		1,067	28	.030
Brood	III		100		3,422	28	.008
Totals			742		14,480	204	.014

TABLE II—A table showing the total number of visible abnormalities in the three experiments classified into sterile, somatic and germinal mutations

		Total No.	Sterile	Somatic	Germinal
			Experiment I		
Brood	I	16	13	3	0
Brood	II	6	0	5	1
Brood	III	10	4	5	1
			Experiment II		
Brood	I	52	21	23	8
Brood	II	16	7	8	1
Brood	III	7	1	5	1
			Experiment III		
Brood	I	41	13	12	16
Brood	II	28	14	7	7
Brood	III	28	7	13	8
Totals		204	80	81	43

TABLE III—A list of the 43 germinal mutations found in the first three experiments, arranged to show the number of times each occurred and their distribution between the X-chromosome and the autosomes.

X-Chromosome Mutants		*Autosomal Mutants*	
Miniature wing	1	Delta (large)	1
Bar to round eye	5	Delta (small)	1
Notch wing	2	Delta (very small)	3
Edge wing	1	Branched	8
Cut wing	1	Outstretched	1
Small wing	1	Curly	1
Truncated	1	Plexus	5
Rudimentary	1	Wrinkled	1
White eye	2	Notch	1
		Vicinal	1
		Blistered	1
		Uneven edge	1
		Crumpled	1
		Minute bristles	1
		Angle	1
Total	15	Total	28

TABLE IV—A table showing the number of visible abnormalities arising among several thousand flies treated with 150 mg. of radium, using a lead filter .005 inches in thickness

Total visible departures from the normal	123	Notch	X-Chromosome
Sterile	55	Outstretched	Lost
Somatic	62	Roughoid	Lost
Germinal	6	Eyeless	4th Chromosome?
		Cream Eye	Autosomal
		Cream Eye	Autosomal

Criminal Tendencies in Identical Twins

LANGE, JOHANNES, *Verbrechen als Schicksal* (Crime as fate). 96 pages, 5 figures. Price 7.00 marks. Verlag Georg Thieme, Leipzig, 1929.

IN the field of human genetics the study of twins in recent years has attracted increasing interest on account of the fact that a comparison of identical and non-identical twins furnishes a valuable criterion for the distinction of inherited and environmental factors. The book by Lange probably is one of the most valuable contributions to this problem. The author, who is a member of the German Institute for Psychiatric Research (Kaiser Wilhelm Institute) in Munich, has studied the life of pairs of identical and non-identical twins of which one was in prison at the time. There were thirty pairs of twins in all. Of the thirteen pairs of identical twins in ten cases both members of the pairs had been in prison, whereas among the seventeen non-identical pairs only in two cases had both members been in prison. The criminality in non-identical twins was not higher than that of ordinary brothers and sisters. The detailed reports about the life of the identical twins, on the other hand, reveal an extraordinary likeness of these twins with regard to their conflicts with the penal code. While the material thus establishes beyond doubt the decisive rôle of the genetic constitution in human conduct, it also shows that other factors (environment, disease) must not be neglected in the analysis of such cases. Lange gives a very interesting discussion of the relation between genetic constitution and other factors with regard to their importance in the causation of crime. Further work of this kind is of the greatest importance and should lead to a revolution in the popular concepts of "crime" as well as in the treatment of those born to be criminals.

WALTER LANDAUER.

PARALLEL CYTOLOGY AND GENETICS OF INDUCED TRANSLOCATIONS AND DELETIONS IN DROSOPHILA

Cytology by T. S. PAINTER,
Genetics by H. J. MULLER
University of Texas

WHEN chromosomes are exposed to X-rays in appropriate doses they exhibit a marked tendency to break in one or more places. As a consequence of this breakage two or more fragments are formed. These may remain detached but they very often unite at their broken surfaces with other chromosomes or chromosome parts. (The sequence of events here is uncertain; it may be that the attachment usually occurs first and that the breakage is a secondary consequence of this; evidence on this point is not at hand). A detached fragment or a combination of fragments if fiberless will fail to be properly transported at mitosis and hence will eventually be lost, but when it has a spindle fiber attachment it can persist and be transmitted to subsequent cell generations (e. g., the cases of the "deleted X" and of "Tubby," described below). There is reason to believe that if a combination chromosome is formed which has two points of fiber attachment this also will tend to be lost (presumably through being pulled in two directions at some of the mitoses), especially if the points are far apart; such cases have not yet been encountered in our work.

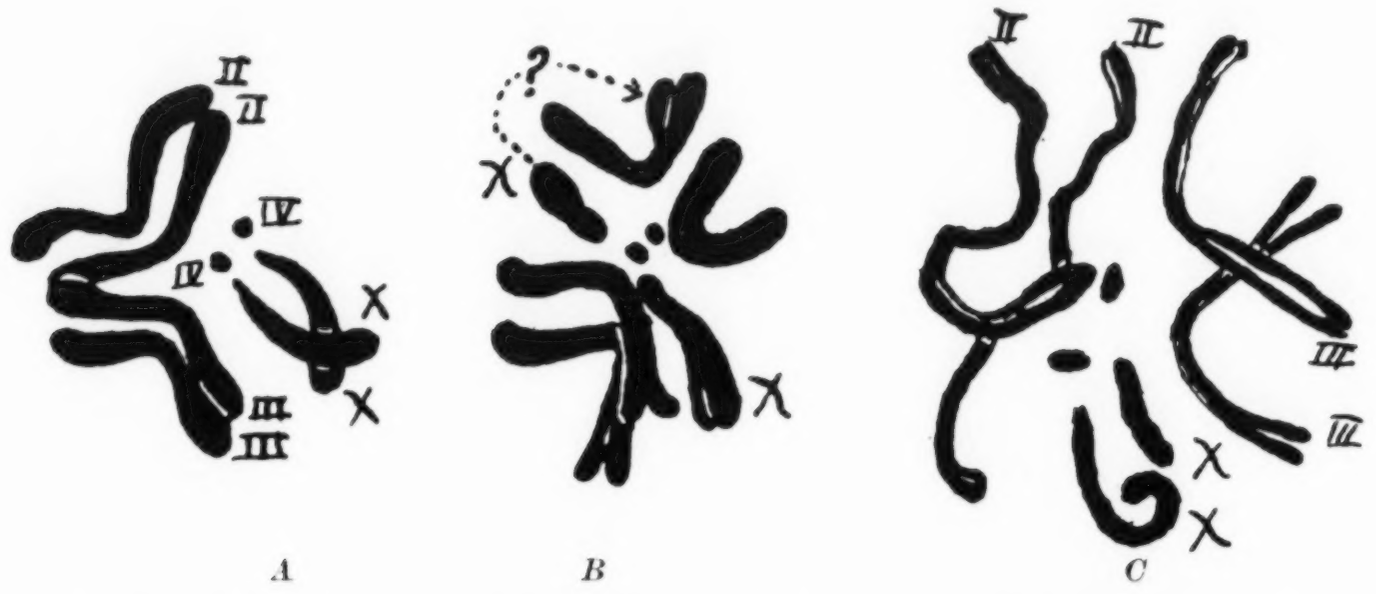
Sometimes the fragments of the same chromosome both or all reunite but in a different order than before (inversion). In other cases two terminal fragments unite together leaving out a median section (deletion). Again, one of the fragments may become united to a homologous (or sister) chromosome (duplication) or to a non-homologous chromosome (translocation). All four of these latter transformations have been realized in experiments carried on at this laboratory. There are also other, more complicated possibilities. The present paper will deal largely with the genetic and cytological evidence for the translocations and deletions which the writers have been studying cooperatively. In a separate paper Muller and Altenburg will describe the frequency of these translocations following irradiation and the genetic methods by which they are detected.

In Figure 27*A* the normal chromosome complex of Drosophila melanogaster is shown*. We have found both genetically and cytologically that the *X*, the second, and the third chromosome may undergo breakage following irradiation and that the fiberless fragment resulting may be attached to any of the other chromosomes present, including chromosome IV and the *Y*. Elsewhere† we have given figures of these various types of translocations together with an outline of the genetic findings.

*To those unfamiliar with the chromosomes of *D. melanogaster* a word of explanation is here given. There are four pairs of chromosomes, which have been given numbers, as shown in the drawing. The *X* chromosome is I, and tends to appear as a more or less straight tapered rod. Chromosomes II are somewhat larger than III, and the IV's are very small. Characteristically the mates lie side by side as shown in the figure.

All drawings of chromosomes were made with the aid of a camera lucida. All figures were taken from ovarian tissue following a special technique for the preservation of Drosophila chromosomes developed by one of us (T.S.P.).

†In the *American Naturalist*, Vol 63:193-200 (1929).



NORMAL CHROMOSOMES AND TRANSLOCATIONS

Figure 27

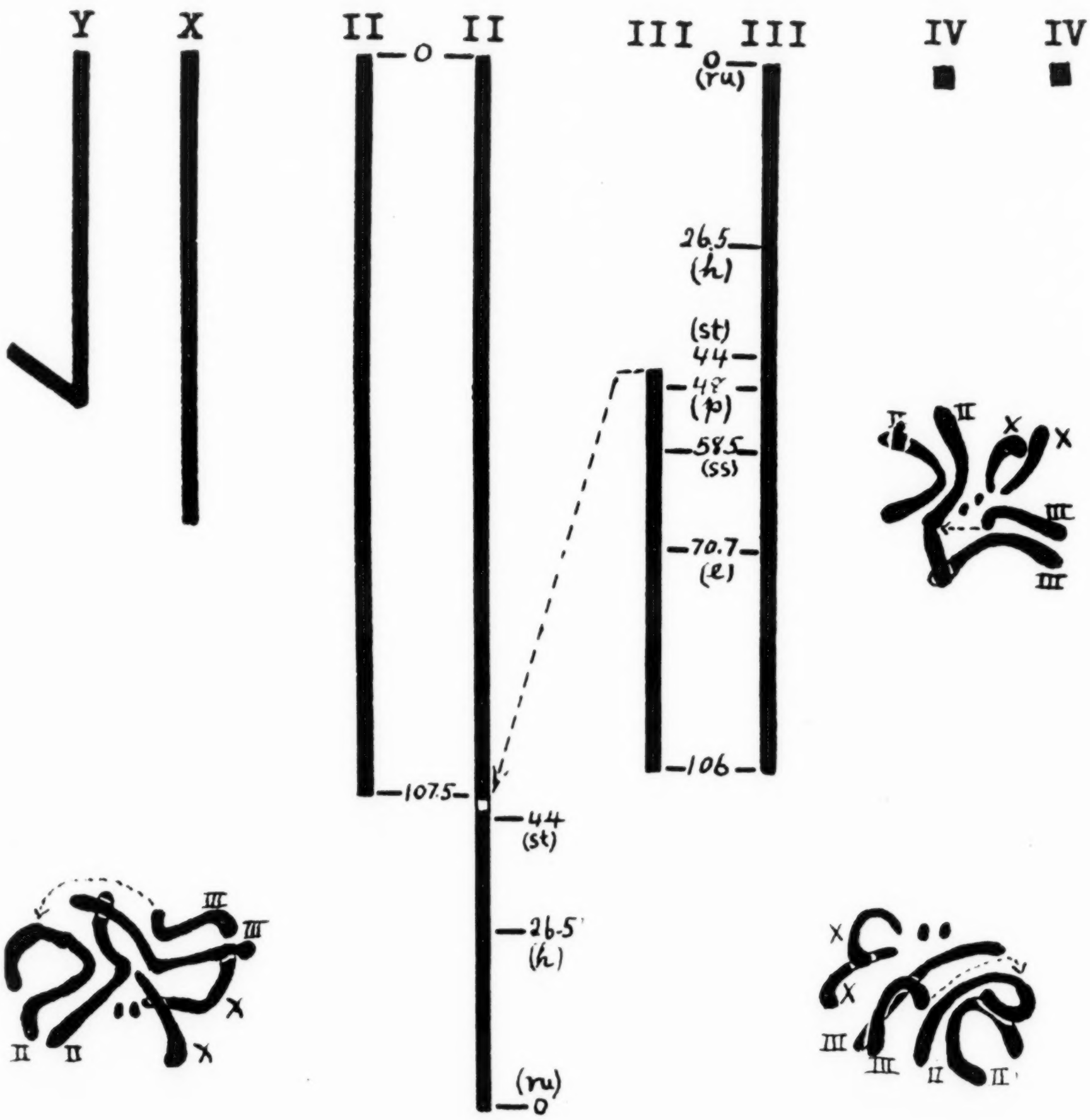
A represents the normal chromosome complex of *Drosophila melanogaster*. For further explanation see footnote on preceding page. *B* and *C* show that one X chromosome is much smaller than the other following a translocation. In *B* it looks as if the broken piece were attached to one of the third chromosomes, but in *C* this relation is not apparent.

Here we shall confine our attention, with the exception of one case of historical interest, to a series of breakages in the third chromosome since this group of cases adequately illustrates the character of the evidence, genetic and cytological, and also brings out the features of these cooperative translocation studies which bid fair to be of the most general interest.

Figure 27*B* and *C* illustrates the first translocation discovered in the X-ray work on Drosophila (December, 1926). The cytological work in this case was done by Muller, and the preparations were described and demonstrated by him at the Nashville meeting, December, 1927; the figures are of interest because this was the first microscopical demonstration in Drosophila of a chromosome breakage known to be associated with a genetically determined translocation. In this instance one of the *X* chromosomes has broken and, as Figure 27*B* suggests, the fragment appears to be attached to an arm of one third chromosome. In Figure 27*C*, which represents an earlier prophase, the difference in size of the two *X* chromosomes is again obvious, although it is not clear to which autosome the fragment is attached.

Figure 28 epitomizes the genetic and cytological data concerning our "Star-Curly" translocation. No explanation is needed except to point out that the genetic evidence shows, first, that the third chromosome is broken between the locus for scarlet and pink; second, that this piece is attached to the "right hand" arm of the second chromosome (the lower arm, as conventionally shown in vertical position), and third, that the broken surface, not the distal end of the fragment, is joined to the latter. Genetic evidence is lacking as to just where on the right arm the fragment is attached (due to the suppression of crossing over caused by the inversion accompanying the contained gene for curly wings) but this information is supplied by an examination of the chromosomes themselves.

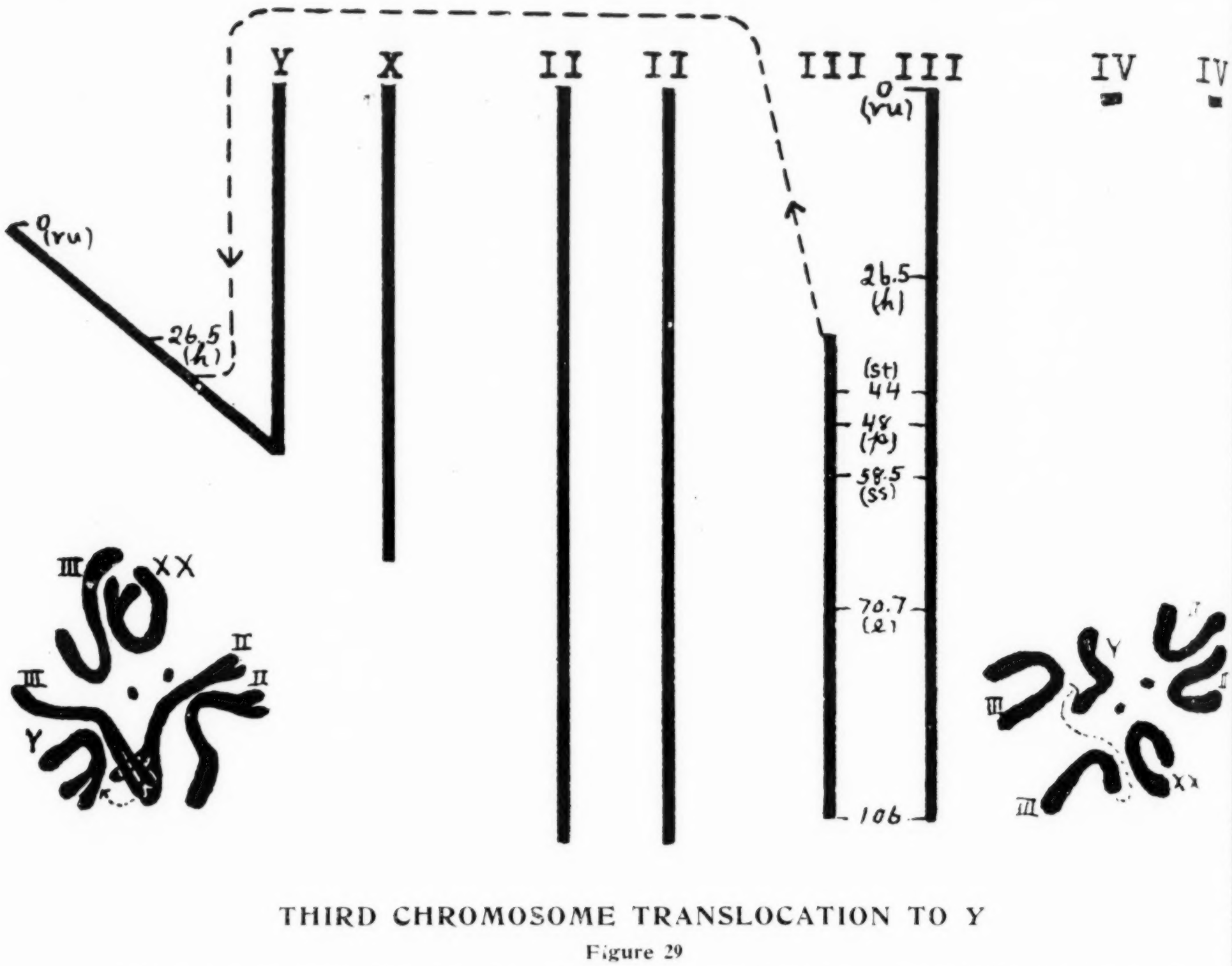
Figure 29 gives the combined results for "translocation III to *Y* 1." Here the genetic data show that the break of the third chromosome has come between the locus for hairy (26.5) and scarlet (44.0). In making up the genetic map we have indicated the point of breakage as midway between these two loci. The genetic evidence further shows that the smaller fragment is attached somewhere to the *Y*. The cytological evidence likewise shows that the third chromosome is broken, and that its smaller (fiberless) piece is attached to the *Y*, but it seems to indicate that the attachment is to the short arm of the *Y* chromosome, so as to make this element a small V with arms of almost equal



THE "STAR-CURLY" TRANSLOCATION

Figure 28

The combined genetic and cytological findings of our "Star Curly" translocation are epitomized here. One of the third chromosomes has broken between the locus of scarlet (*st*) and pink (*p*) and has become attached to the lower "right-hand" end of a second chromosome (see maps). In the drawings of the oogonial chromosomes, it is seen that almost the whole of one arm of one of the third chromosomes is missing, and that one of the second chromosomes is much longer than normal. The arrows in this and subsequent drawings show the point of break and the point of new attachment. The mutant genes indicated in parenthesis in the genetic maps of the translocations were in each case tested in connection with the translocation with which they are shown; the chromosome which underwent translocation in each case here shown originally carried the normal allelomorphs of these genes. It is not to be understood that the mutant (rather than the normal) allelomorphs are necessarily present, when they are represented in *parenthesis*.



THIRD CHROMOSOME TRANSLOCATION TO *Y*

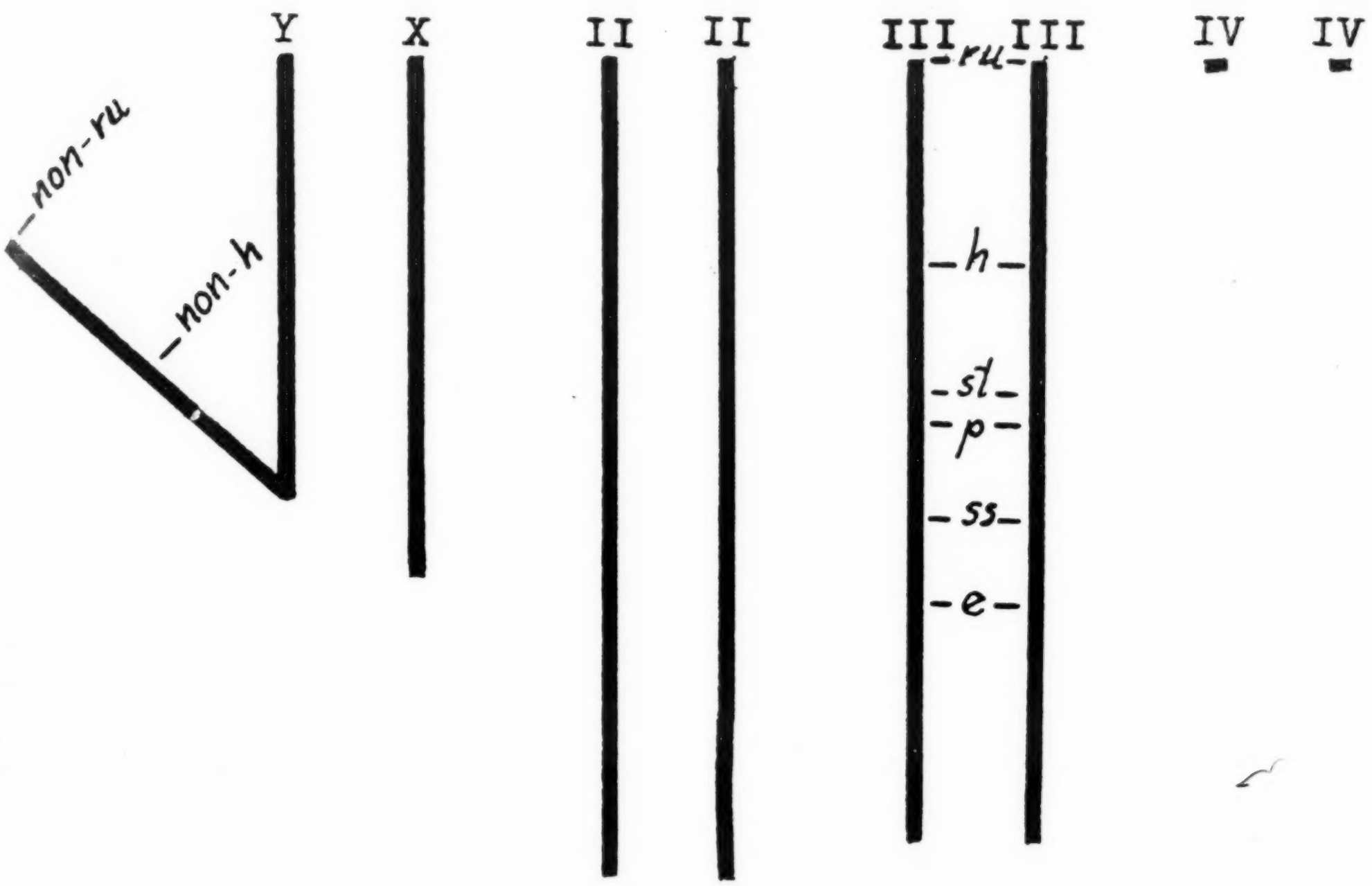
Figure 29

This figure presents data for the "III to *Y* 1" translocation. Genetically we know that one of the third chromosomes (see maps) has broken between the locus for hairy (*h*) and scarlet (*st*) and has joined to the *Y* chromosome. Cytologically we see in the two oögonial figures given, that one of the third chromosomes has lost chromatin from one of its ends and this seems attached to the short arm of the *Y* chromosome, making it a small V.

length. We do not know by which end the fragment is attached, but in the maps we have represented the broken surface as joined to the short arm of the *Y*, since from other considerations this appears to us as probable.

Figure 30 represents the genetic evidence for hyperploidy of the fragment of the third chromosome involved in the above translocation (III to *Y* 1). The condition shown was produced by introducing the *Y* chromosome carrying this translocation into an otherwise normal chromosome configuration, in which the third chromosomes carried the recessives indicated. (The fly therefore exhibited the characters due to non-*ru* non-*h* *st* *p* *ss* *e*.) This case is of interest because the hyperploid flies of this chromosome configuration, even when carrying no mutant genes whatever, are phaenotypically different from the normal, showing short bristles, broadened convex wings with imperfect cross veins, misshapen eyes, melanism, incurved hind tarsi, and other peculiarities.

Figure 31 describes translocation "III to II 26", which has been analyzed fully genetically and which is of especial interest because the translocated piece is not nearly so large cytologically as we might expect from the genetic data. The break has occurred

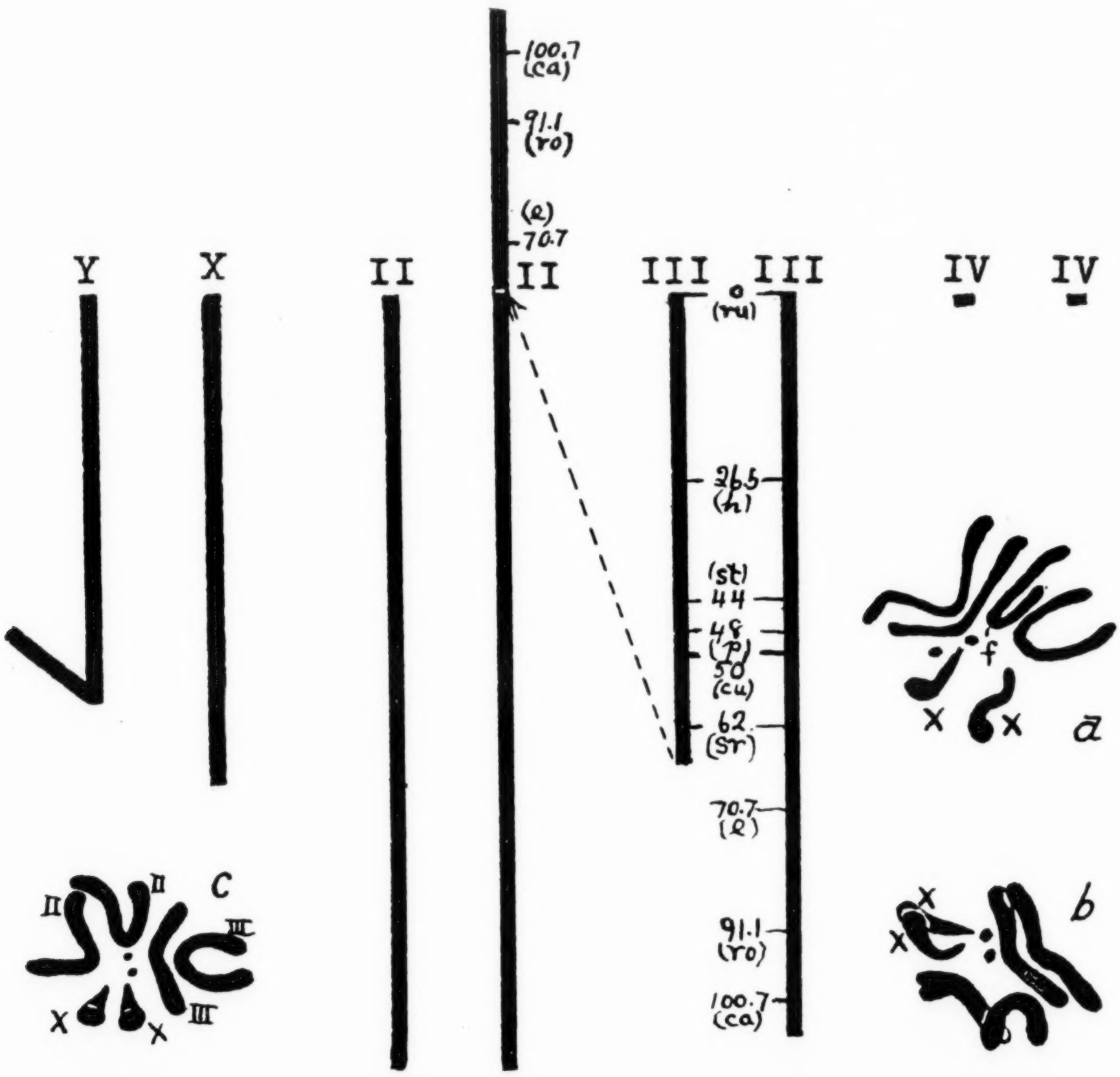


MASKING EFFECT OF THIRD CHROMOSOME FRAGMENT

Figure 30

Chromosome maps showing the genetic constitution of hyperdiploid flies which carry attached to their *Y* chromosome the segment of the third chromosome described above in Figure 29. In the flies that have been obtained having the genes shown in the diagram (fig. 30), the dominant normal genes in the added fragment prevent the appearance of the corresponding recessive mutant genes present in the homozygous third chromosomes, but the other recessive mutant genes in the third chromosomes, not "covered" by the translocated piece, are able to manifest themselves. Hence these flies show non-*ru* non-*h* *st* *p* *ss* *e*.

between the locus for stripe (62.0) and ebony (70.7) and thus involves the loss by the third chromosome of at least 35 genetic units, a third of its length. The genetic data further show that this right hand or lower fragment of the third is attached to the left hand or upper end of the second chromosome, and that the point of attachment is at the broken end, not the originally terminal end, of the fragment. When we examine the chromosomes themselves the piece involved in this translocation is small and is not readily detected by inspection in condensed stages. Of the three cells illustrated in Figure 32, *A* is a prophase stage which shows a difference in the size of the mates of the two pairs of large autosomes. For example, in the case of the pair to the left the outer is longer than the inner, the order of difference being about a sixth to a tenth of the total length. One arm of the right-hand pair is foreshortened (f) but even when this is taken into account it is shorter than its mate. We can not be certain in this cell which is the donor or third chromosome and which the recipient or second chromosome, but it is clear that there has been an exchange involving a piece much less than a third of a chromosome. The cell labeled *C*



A "'III TO II" TRANSLOCATION

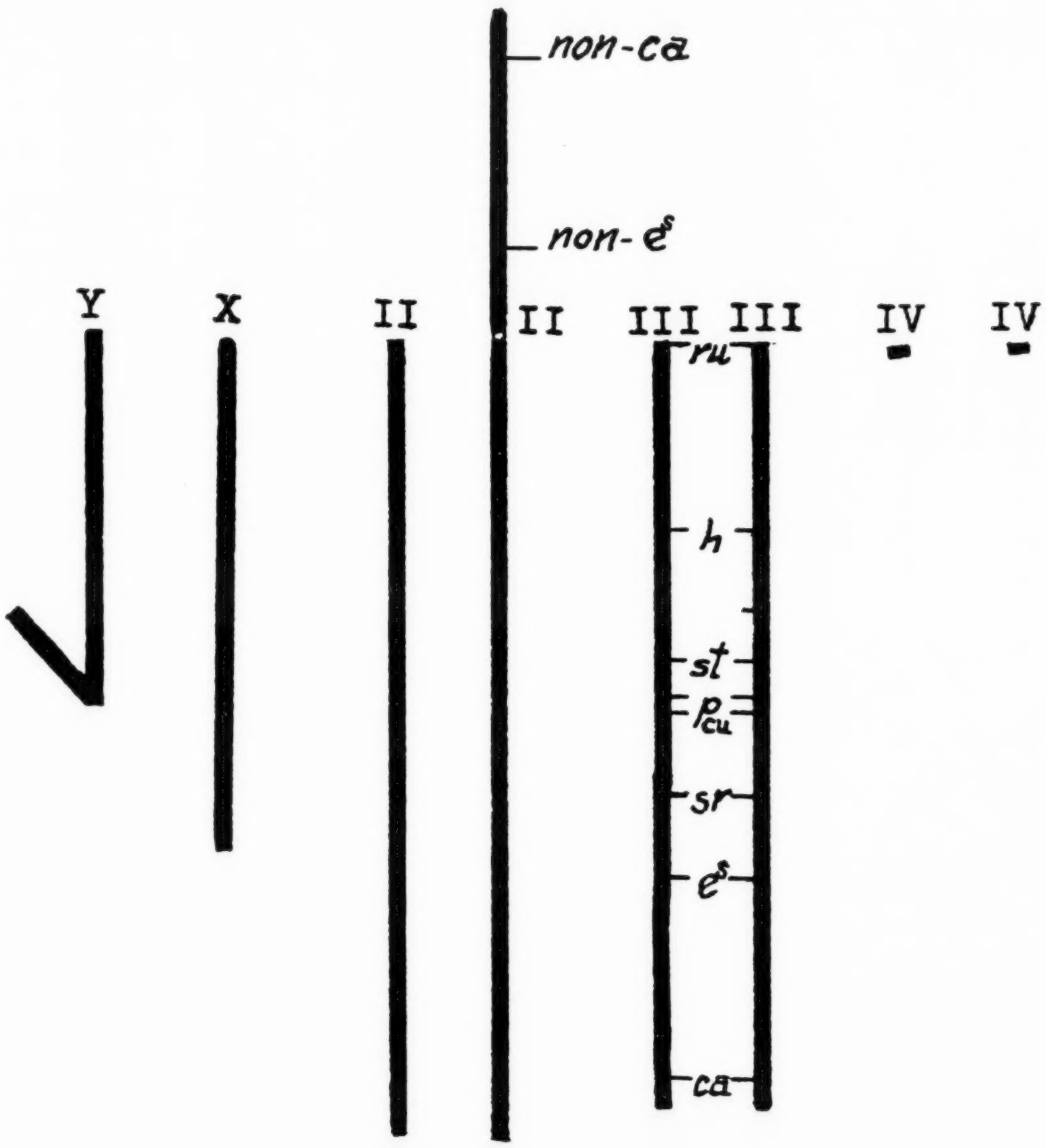
Figure 31

This figure represents conditions encountered in translocation "III to II, 26." In this case it is the "right hand" end of the third chromosome which has broken and become attached to the "left hand" end of the second chromosome. Although fully a third of chromosome maps as given is involved here, when we examine the chromosomes themselves the size of the translocated piece is found to be small. In *A* it is clear that the mates of the two large autosomes are of unequal length. In the case of the pair to the right one chromosome (labelled *f*) is foreshortened, which makes it appear much smaller than it really is.

is a condensed stage in which the second and third chromosomes can be identified. On inspection it is not obvious that the inner of the third chromosomes is the shorter, though this is found to be the case on measurement, and of the second chromosomes we are not sure which is the longer.*

By making the appropriate crosses the second chromosome carrying the piece of the third illustrated in Figure 31 can be introduced into an otherwise normal configuration. The genetic map of this hyperdiploid is shown in Figure 32. When supplied with genes as shown it was found to exhibit the

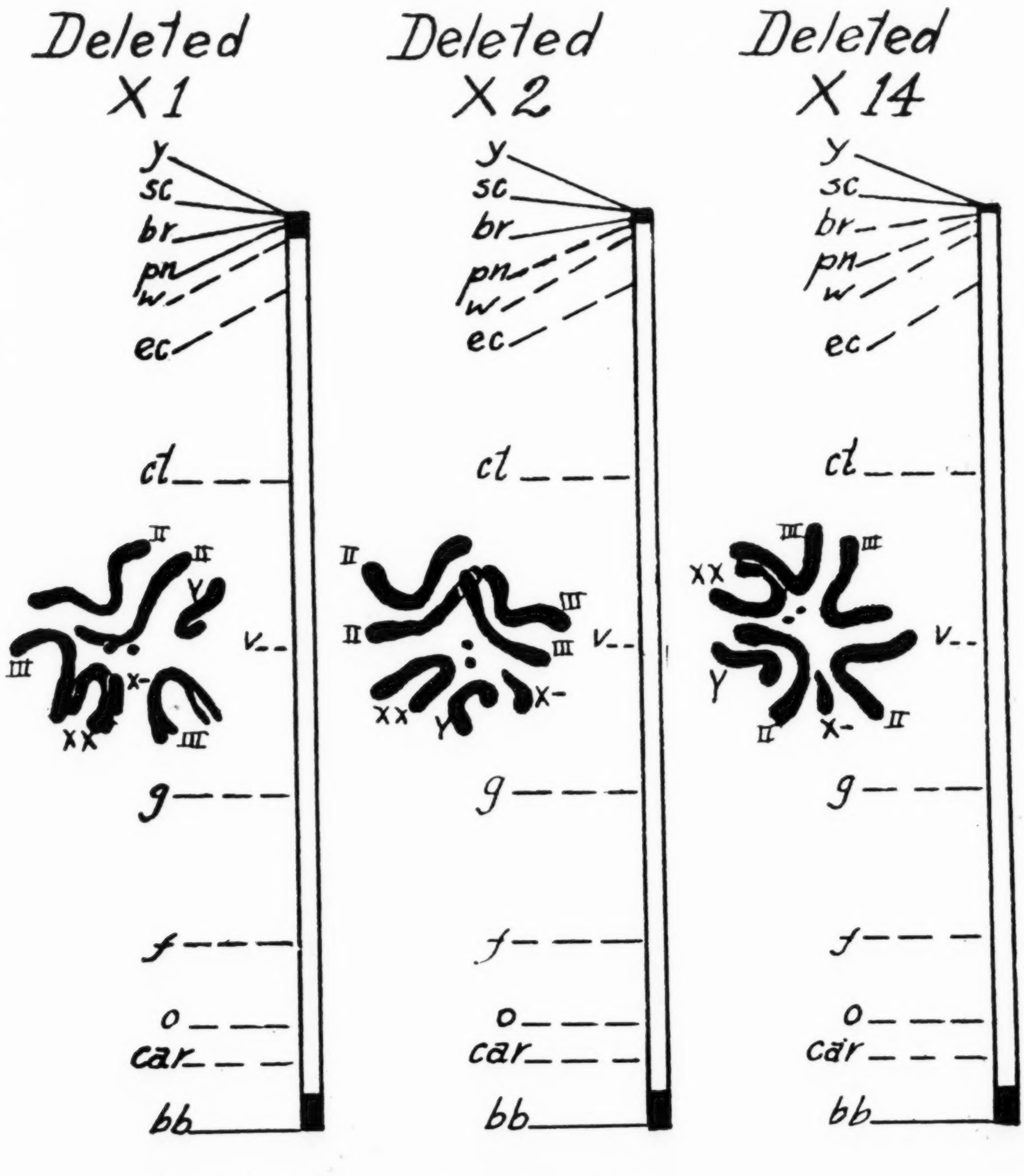
*A diagram based on cytological preparations of Muller, which showed this translocation, was exhibited by him at the meeting of the National Academy of Sciences in Washington, April, 1928, in connection with his paper, "The Production of mutations by X-rays."



HYPERDIPLOIDY PRODUCED BY ADDITION OF TRANSLOCATED CHROMOSOME

Figure 32

This figure gives the genetic constitution of hyperdiploid flies produced by introducing the second chromosome carrying a piece of the third, illustrated in figure 31, into an otherwise normal complex. In the flies of this composition that have been obtained we find, as in the hyperdiploid of figure 30, that the dominant normal genes in the added fragment interfere with the (full) expression of the recessive mutant genes present in the homologous portions of the third chromosomes. Thus the flies fail to show *ca* (claret eyes) at all, while in the case of e^s (sooty body) it is found that one "dose" of the normal gene, non-e^s (representing grey body color) partially dominates the two doses of e^s, producing a body color of intermediate darkness. On the the other hand, the recessive mutant genes to the left of the point at which the fragment was broken off are not "covered," and manifest themselves fully. Thus the appearance is *ru h st p cu sr* interm-e^s non- *ca*.



"DELETED *X*" CHROMOSOMES

Figure 33

This figure summarizes our knowledge regarding three cases of deleted *X* chromosomes. The maps are drawn to the usual to scale and the black portions represent those portions of the chromosome which were present genetically. The loci here indicated by solid lines were present, and in each case carried normal allelomorphs except in the case of bobbed of deleted *X*1 and *2*, which had mutant allelomorphs of intermediate degree of expression. The loci indicated by dotted lines were tested for but found absent genetically. To the left of the map in each case the deleted *X* chromosome is indicated by *X*- in the drawings of the chromosomes.

characters due to *ru h st p cu sr* e^s-e^s-non-e^s (intermediate) and non-*ca*. It differs from normal even when it contains no mutant genes. Its peculiarities are divergent wings, imperfect fifth longitudinal veins, elongated bristles and small eyes.

Deletions of X Chromosomes

Hyperploidy of a part of a chromosome may be produced in several different ways from flies treated with X-rays. The cases described above illustrate one method, based upon translocations. In practice, however, we have obtained more readily hyperdiploids of a different origin, based upon deletions of the *X*. These have been produced by irradiating adult males and then crossing them to females of the attached *X* race. When this is done, in approximately one per cent of the cases, with our customary heavy dosage, the *X* chromosome from the male undergoes a deletion of its middle part, leaving a small piece from the left-hand end attached to a small fragment of the right-hand end which bears the spindle fiber. Such a chromosome, in conjunction with the attached *X's* bearing yellow, results in a non-yellow (grey) female which is not as markedly abnormal as the grey triple-*X* females which also hatch occasionally in these cultures. Various deletions of the *X* chromosome of this general type have been analyzed, and it has been found that the size of the surviving fragment varies genetically and cytologically. Three cases have been selected for illustration here, the combined results on these being presented in Figure 33. Here the *X* chromosome is shown to usual scale and the heavy black portions represent the loci which are present in the deletions. Thirteen loci were tested genetically in all these cases. Nearest the left-hand (upper) end these were yellow, scute, broad, prune and white, in this order; nearest the right-hand (lower) end, carnation and bobbed. Deleted *X* 1 carries the loci for yellow, scute, broad and prune (has normal allelomorphs of these) but as the dotted line indicates the locus of white is not present in the left-hand end. In the right-hand end the locus of bobbed is present (there being a mutant bobbed allelomorph of intermediate degree of expression here, as had been the case in the chromosome prior to its deletion); on the other hand, carnation (at 65.5) is definitely absent, and none of the intervening loci are represented. Deleted *X*2 carries the normal allelomorphs of yellow, scute and broad, but does not carry the loci for either prune or white, or for any of the genes tested to the right of these, until we reach bobbed (where an intermediate allelomorph is represented, as in Deleted *X*1). Deleted *X*14 has the normal allelomorphs of yellow and scute, but shows no loci beyond these in the left-hand region; all the intervening loci, too, are missing, but the normal allelomorph of bobbed is present at the right-hand end.

It will be observed that in all these cases one coherent section of the genetic map has been deleted, including every one of the genes tested in that section, while in the remainder of the chromosome all the genes, without exception, are still present. This illustrates the fact that the genes which the map represents as lying together really do lie together physically. More detailed evidence is provided by a comparison of the left (upper) ends of the different deletions. For, whenever the scute locus is present, that of yellow is present, though the broad locus—which is shown to the right (below) in the map—need not be present, being absent in deleted *X*14. The fact that broad became detached from the former loci this way when the chromosome broke shows that it cannot be *between* yellow and scute in the actual chromosome, and must therefore be beyond (to one side of) them. Secondly, we find that when the broad locus is present, both those of scute

and yellow are present, but that of prune need not be (deleted *X*2). Hence, by the same method of reasoning, prune is physically beyond yellow, scute *and* broad in the chromosome. Thirdly, when the locus of prune is present, those of yellow, scute and broad are present, but that of white need not be present (deleted *X*1). This places white beyond prune. These deletions, then, give direct evidence that the four loci—for scute, broad, prune, and white, respectively—lie physically in the order just named, which is exactly the same as the order in which they are shown on the map. We have analyzed other breakages of the *X*, some of them still farther to the right, and in each case the principle of map validity holds in this way. (Note too its application in the case of the translocations above pictured). When coupled with the cytological evidence that in these cases a piece of the chromosome is physically missing or removed, it constitutes direct proof that the genes in general lie in the same actual sequence as the standard genetic map (hitherto based only on crossover relations) has pictured them to lie in.

In the case of the deletions now under consideration, drawings of the chromosomes have been presented with the maps, the deleted *X* being indicated as "*X*—." The small size of these chromosomes, as compared with undeleted *X's*, is clearly seen in these figures. The undeleted *X's* are in these cells attached to each other, since it will be remembered that in the crosses in which the deletion was produced the irradiated males were crossed to females of the attached X type; this also explains the presence of the *Y* chromosome in these figures. But although the figures obviously contain an abbreviated chromosome, as required, this chromosome is in no case nearly as short, relatively to the other X's, as the genetic maps would lead us to expect, if taken at their face value. Cytologically the deleted *X's* are at least a quarter the normal size, whereas the map shows them not more than a twelfth. This point will be referred to later.

The presence of a deleted *X* in addition to either one or two normal *X's* gives rise to certain characteristic phaenotypic abnormalities, which vary somewhat according to the exact loci included in the deletion. The bristles are coarse, often duplicated, and the hairs rather abundant; the wings tend to be divergent and narrow and sometimes have branched veins, and the abdomen is somewhat blunt. These abnormalities result from an unlikeness between the dosage of genes in different parts of the *X*-chromosome ("intra-chromosomal genic unbalance"), since of course a double dose of all genes in the *X* produces only a normal female. Since the males can breed, it is also evident that the sex-differentiating gene or genes are not contained in the deleted X.

An Extra Linkage Group

We have found one case of a hyperdiploid which agreed with those described above in carrying as excess a free spindle-fiber-bearing piece of a chromosome, but in which the flies carrying the fragment were of a different type phaenotypically from any carrying a deleted *X*. They have been named "Tubby," and are characterized by a much shortened broad body, bulging eyes and somewhat divergent wings. The variant arose after raying as a dominant "mutation," but when tested proved to lie in none of the known linkage groups and so constituted a fifth group of its own. Here then was the case of apparent breakdown of the chromosome theory of heredity for which some of its opponents had long waited. The case was then submitted to the cytologist, who demonstrated within half an hour after examining a section of an ovary, that it

ADDED FRAGMENT IN "TUBBY" "MUTANT"

Figure 34

Here is shown the presence of an extra chromosome in the hyperdiploid fly "Tubby." The genetic analysis of this fly indicated a fifth linkage group, and cytological examination disclosed the reason for this in the fragment indicated by the arrow.

was a chromosome which had broken down, not the chromosome theory!

The examination of the chromosomes showed the presence of a very small chromosome fragment in an otherwise normal complex (Figure 34). The size of this fragment is only about six times that of one of the fourth chromosomes, near which it usually lies. It is probable from genetic evidence that this fragment was once a part of one of the large autosomes, but it is so small that the genes it carries have not yet been found. Genes in the neighborhood of the spindle fibers of each of the four known linkage groups have been tested and found absent from it, as also are the genes of the left-hand end of the *X*.

Value of Translocation and Deletion in Research

In presenting the genetic and cytological facts of just a few cases of the many which we have worked out together we have shown the nature of the evidence for translocations and deletions. We may now briefly consider the value which these chromosome disturbances and rearrangements have for the biologist. Undoubtedly, translocations will prove to be a new and very effective tool in the further analysis of genetic and chromosomal phenomena of various kinds, as we have pointed out elsewhere.* Aside from furnishing new and critical evidence for the theory of the linear arrangement of the genes, perhaps the point of greatest general interest is the promise which translocations and duplications give of allowing us eventually to actually plot the spacial relations of the genes in the chromosomes, and to correlate this with chromosome morphology and behavior. It is perfectly clear, from facts now on hand, that chromosome maps based on the frequency of crossing over do not give us an adequate idea of the relative distances of the genes in the chromosomes, in spite of the fact that they represent the order (sequence) of the genes correctly. On the one hand, we have translocations—such as "III to II 26"—involving the transfer of some 35 genetic units and in which nevertheless neither the loss by the donor nor the gain by the recipient chromosome is as evident as we should expect when nearly a third of a chromosome genetically is involved. And, on the other hand, in the case of the deleted *X* chromosomes, as illustrated in Figure 33, although only a few units of the chromosome map are represented out of the 70 present in the normal *X* chromosomes, actually these deleted chromosomes are of considerable size.† It now appears that through a combined genetic and cytological study of these translocations, deletions, etc., by the methods outlined in this paper, we shall eventually be able to construct truer chromosome maps, which will

*See footnote on page 287.

†The results correspond with Muller's earlier conclusion, based on the "crowding" of X-ray and other mutations in the "left" end of the X chromosomes and the central regions of the autosomes, that these regions are really far longer than the map indicates; in the accompanying paper by Harris the crowding effects in the X stand abundantly confirmed (see figure 35 p. 300). That method of attack on the point here at issue—the validity of the map proportions—is, however, relatively indirect as compared with the methods here presented.

show not only the order of the genes but their actual spacial relations, in chromosomes having the appearance seen under the microscope.

There is one more question which may be mentioned here. Is there any evidence that translocations may have played a rôle in the evolution of chromosome configurations? At the present time this is strongly indicated by several different lines of investigations among both animals and plants. For example, one of us (T. S. P.)* on comparing the chromosomes of the rat and mouse reached the conclusion several years ago that while the amount of chromatin for the two species was about the same and the chromosome number nearly identical (42 and 40 respectively), nevertheless the distribution of the chromatin among the chromosomes was very different. It was concluded at that time that since the rat and mouse must have arisen from some common stem there has been a great deal of shifting of chromatin (translocations) between non-homologous chromosomes. The discovery that such translocations, of varied kinds, do occur as a result of absorption of short wave-length radiation (which exists widely dispersed in nature) lends strong support to such an interpretation, and would tend to lend it a more general significance in the evolution of species.

*Painter, T. S., 1928. A comparison of the chromosomes of the rat and mouse with reference to the question of chromosome homology in mammals. *Genetics* 13:180-189.

A World Guide to Animal Breeders

Early in 1929 there was published in England a volume that should be of considerable value to breeders and experiment stations throughout the world*. The list of breeders in the British Isles and the British Colonies must be nearly complete, as in England alone the names and addresses are listed of 8,000 Breeders of Cattle, 3,600 Breeders of Sheep, 4,000 Breeders of Pigs, and 5000 Breeders of Horses. Under each country there is a discussion of the most important breeds, followed by a list of the breeders, so that anyone interested in a particular breed has an enormous amount of information and a large number of names of prominent breeders and of breed associations ready to hand. The book is profusely illustrated with excellent examples of the principal breeds in all parts of the world. For those who require information of this kind the book should prove invaluable as a reference, and the price of $6.00 is remarkably reasonable for a volume that must have involved an enormous amount of labor in its compilation.

*International Directory of Pedigree Stock Breeders: An index of Pedigree Stock Breeders in Great Britain, the Overseas British Dominions, and all Civilised Countries. A Handbook recording Breeds and Stock Breeding, etc., in Every Country of the World. With Maps and Numerous Illustrations. Compiled and Edited by R. de Toll. Published by The Vernon Press, Ltd., 23, Fleet Street, London, E. C. 4, England. 1928-1929.

THE EFFECTS OF AGING OF X-RAYED MALES UPON MUTATION FREQUENCY IN DROSOPHILA

B. B. HARRIS
University of Texas

AMONG the problems brought forth by Muller's discovery (1926-'27) of the production of gene-mutations and chromosome abnormalities by X-rays, was the question of the relative genetic effectiveness of the rays when applied at different stages of the germ cell history[1] and the related question of the possible influence of aging the mature germ cells, upon the production or detection of these variations.[1,2] Conclusive data regarding both these questions, insofar as they concern sex-linked lethal changes in the germ cells of the adult male Drosophila, have been secured by the present author, in a series of experiments conducted during the past sixteen months in the genetics laboratory of the University of Texas, at the suggestion and under the direction of Dr. H. J. Muller. Ten thousand nine hundred and thirty-one F_2 families of flies have been examined during this study, and fifty chromosome abnormalities, twelve semi-lethals and two hundred and one lethal point mutations have been studied by means of crossover tests. Hence it is obviously impossible to present the full details in this preliminary communication. These will be published later, and the following account will meanwhile serve to outline the main features of the work.

The "C1B" method was used throughout in this study. The flies were grown in 1″×4″ glass vials. The X-ray dosage was 30 minutes exposure at 13 cm., 50 K. V. peak, 10 milliamperes, with 1 mm. aluminum filter. The males only were treated, and, in the experiments shown in Tables I, II and III, these treated P_1 males were then immediately mated with non-treated virgin P_1 females, in pairs. Every four days the same P_1 males were placed in a fresh set of vials, with other virgin P_1 females, and the progeny (F_1) derived from the matings in these different periods after treatment were bred, as P_2, in separate cultures, the F_2 families being examined for lethals. Tables I, 11 and III give the data on lethals in these P_2-F_2 cultures, derived from the different periods of matings of the P_1 males. (In the case of Table II, groups of periods are shown, and there is one four-day period in both Table I and II from which F_2 were not obtained.) In the experiment shown in Table IV, on the other hand, different sets of P_1 males were used in the different periods following treatment, the P_1 males mated at any given period after treatment having been kept in isolation from females until that time. The contrast between these results and those in the first three tables is obvious. It may be added that in continuation of this latter experiment treated males are now being held for a still longer period—32 days—before mating.

In the case of the experiment shown in Table III, the different P_1 males were given separate identification numbers, so that the output of lethals by the same male in different periods could be compared, in contrast with that of other males. The lethals that arose in this experiment were also subjected to further testing by means of crosses of the appropriate F_2 flies, carried out in half-pint bottles, and the numbers of non-crossovers and crossovers of different classes appearing among the F_3 were counted. Thus it was possible to distinguish chromo-

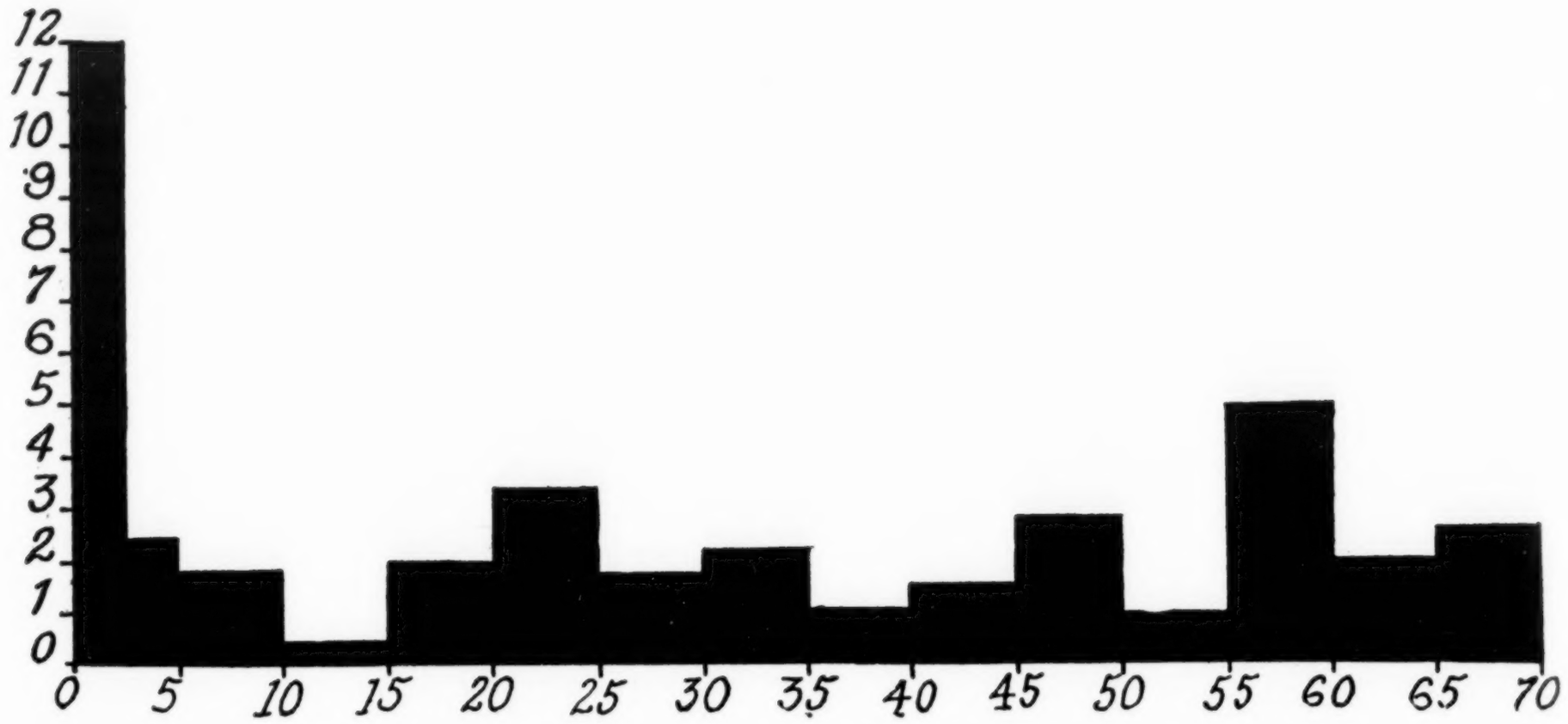


Figure 35

Distribution of 175 lethal mutations in each five-unit section of the chromosome, comprising those cases in which more than 50 F_2 males were counted, showing the variation in mutation rate in different parts of the chromosome. The "peaks" represent regions relatively longer than they are shown on the standard maps, in which crossing over is comparatively infrequent.

some abnormalities from point mutations, and to determine the approximate loci of the latter. The determination of these loci made it possible, among other things, to ascertain whether or not, in any of the periods, groups of identical mutants might occur, which had been derived by subdivision of one original germ cell present in the treated P_1 male. (See summary, point 2).

A graph showing the number of lethals thus found, in each 5-unit section of the chromosome (or 2.5 units in the case of the first two sections), is shown in Figure 35. The distribution of the mutations here evidently agrees strikingly in all its main features with that depicted by Muller on the basis of his experiments of 1926-'27,[2] and indicates differences in crossing over frequency in different parts of the chromosome. Thus the "peaks" represent regions relatively longer than indicated on the standard maps, in which crossing over is comparatively infrequent, and the "troughs" regions of the opposite character, possibly correlated with the places at which the chromosome, as cytologically observed, shows a tendency to bending and constriction.

The following facts emerge from a careful study of the data concerning the effects of "aging" the treated male germ cells on the frequency of sex-linked lethals:

1. The percentages of mutations found are sensibly identical when the treated mature spermatozoa have been aged zero to four days, four to eight days, or sixteen to twenty days (Table IV). There is therefore (within these limits) no correlation between the production of mutations in spermatozoa and their viability or the duration of their functional capacity. From the above it also follows that the mutational effect of X-rays upon the mature sperm probably is permanent during the life of the sperm.

2. The testes of the adult male Drosophila contain not only mature spermatozoa, but also immature germ cells, some of which undergo repeated divisions in the adult testis before becoming mature. (a) This is proved by the grouping of mutant progeny from the treated males in the case of all the periods subsequent to twelve

days, there being much more of a tendency for plural mutant progeny (i. e., more than one mutant from the same male) in these periods than a random distribution of mutants among the progeny of the different males would allow. (b) It is further proved by the sensible identity of locus of the lethals in any given batch (from the same treated P_1 male) in these periods. (c) The figures concerning this grouping show that a single primordial germ cell, present in the adult testis at the time of treatment, multiplies during the ensuing two or three weeks to form, on the average, about half of all the mature spermatozoa then to be found in that testis. Therefore, the proliferation of germ cells in the testis probably occurs through a system of one or a very few indefinitely reproducing cells functioning like apical cells, in that they give off, at each division, one cell like themselves and one other daughter-cell which is destined to redivide only a limited number of times.

3. (a) X-rays are far more effective in producing transmissible gene mutations and also chromosome abnormalities in the mature spermatozoa than in the immature male germ cells. This is illustrated by the fact that the frequency of mutations is five to ten times greater in offspring produced during the first twelve days after treatment than during the second twelve days, in the case of males which are allowed to reproduce from day to day (Tables II and III). (b) The drop is very sharp just between these two periods, i. e. after twelve days (at 27° C.). (c) After 36 days the absence of significant effect of the rays seems especially pronounced, there having been no demonstrable effect with the numbers used (Table II). (d) There are two possible explanations for the relative ineffectiveness of X-rays applied to the immature male germ cells; it is not yet possible to say which of these (if not both) is correct. On the one hand, it may be that the mature spermatozoa are especially susceptible to the X-ray action, their chromatin being in a more easily affected condition. On the other hand, it may be that immature germ cells in which zygote-lethal changes have been produced tend to

TABLE I

Spring 1928

(Same males mated with successive females every four days).

Age of germ cells in days after X-raying	No. of F_1-F_2 vials of flies	% Lethals
1-4	538	7.0
8-12	377	4.5
Controls	369	0.3

TABLE II

Fall 1928

(Same males mated with successive females every four days).

Age of germ cells in days after X-raying	No. of F_1-F_2 vials of flies	% Lethals
1-12	2107	4.75
12-24	1130	0.85
28-36	208	0.96
36-48	499	0.0

TABLE III

Spring 1929

(Same males mated with successive females every four days).

Age of germ cells in days after X-raying	No of F_1-F_2 vials of flies	% Lethals
1-4	1206	8.6
4-8	886	9.7
8-12	875	7.3
12-16	960	1.7
16-20	823	0.6
20-24	740	0.8
Controls	617	0.2

TABLE IV—Males isolated from females until final mating

Age of germ cells in days after X-raying	No. of F_1-F_2 vials of flies	% Lethals
Male removed after a single copulation within 1-10 hours after treatment	220	10.0
Males held 4 days without females before mating-4-8 days	189	9.0
Males held 16 days without females before mating-16-20 days	183	9.0

be killed or to multiply much less rapidly than the genetically normal cells in the same testis (a kind of "germinal selection"), whereas in the case of mature sperm, mutations and chromosome abnormalities do not affect the cell viability or functional capacity.[3] (Table IV).

4. The distribution of mutations in different regions of the X chromosome (Figure 35) was found to have the same features as those reported by Muller for the mutations in his experiments. These phenomena, therefore, depend upon some real and relatively permanent structural peculiarities of the X chromosome.

After the above work had gotten well under way, it was found that Professor F. B. Hanson at Washington University, St. Louis, and the present writer at the University of Texas had independently undertaken a similar series of experiments, at least so far as the item numbered "3" above is concerned. A preliminary report of Professor Hanson's results is being given in *Science,* but he has kindly given the present writer permission to say that the results of these two series of experiments are in entire agreement, on those points (covered in item 3) with which both are concerned.

Literature Cited

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3. Muller, H. J. and F. Settles. 1925. The Non-functioning of the Genes in Spermatozoa. *Zeit. ind. Abst. u. Verb.* Sup. Bd. 43:284-312.

Milk Samples Desired for Chemical and Vitamin Determinations

IN connection with an extensive research on sources of vitamins necessary for calcium utilization and for growth, which at this time, is including samples of milk products from about 270 places in the United States, Canada, Cuba and Mexico, weekly, fortnightly or monthly, information is developing which emphasizes the importance of food sources and individual animal characteristics as controlling factors for the vitamins in these dairy products. This study is being carried through a year of seasons and is a repetition of a similar study made during the year 1928 for a more limited area. This stresses the desirability of extending the investigation to different species and varieties. It would be of great assistance if those in a position to do so would send directly to the writer from 25 to 50 cc. of milk into which has been placed boric acid to equal 0.5 per cent. This quantity of milk will be sufficient for both extensive chemical analysis and for determination of several of the vitamins. Of course, if 5 cc. of the butter-fat could be sent, either in addition to or in place of one-half of the above milk, it would be still better. The expense for shipping this milk will be gladly defrayed by the writer. It will be helpful to know the ration that the animal has been living upon and the date the milk was taken, also the eating habits and other characteristics of the animal. It may readily be that some species, varieties or strains have much higher levels of vitamins than our present available sources of dairy products. The matter is at least one of much scientific interest and well worthy of the effort. This information will be published and sent directly to those interested. These samples should be addressed to:

Weston A. Price.

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Cleveland, Ohio.